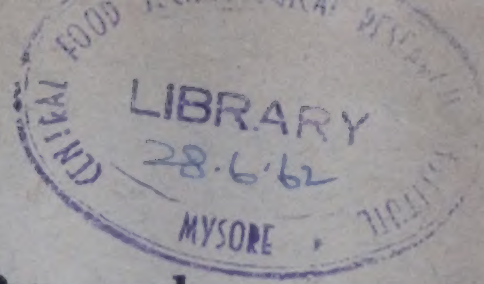
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TECHNICAL REPORT OF THE
SCIENTIFIC ADVISORY BOARD
FOR THE YEAR 1958



Indian Council of Medical Research

TECHNICAL REPORT

OF THE

SCIENTIFIC ADVISORY BOARD

FOR THE YEAR

1958

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Sub-Committee for Compiling Information regarding Facilities, etc., on Physiological Problems in Medical Colleges

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Committee for Developing Standard Methods of Analysis of Water, Sewage and Trade Effluents and Formulating Standards of Quality, Etc.

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Shri K. Venkataramanan, c/o Directorate-General of Health Services, New Delhi.

Dr. K. G. Veeraraghavan, Chief Water Analyst, King Institute, Guindy, Madras. (*Secretary*)

III. TECHNICAL REPORT OF THE RESEARCHES CARRIED OUT DURING THE YEAR 1958

The researches carried out during the year under report were recommended by the Scientific Advisory at its meetings held in Lucknow on the 20th and 21st Dec. 1957 and were approved by the Governing Body of the Indian Council of Medical Research at its meeting held in New Delhi on the 29th March, 1958. *The views expressed by the individual workers are not necessarily the views of the Council.*

CLINICAL RESEARCH

1. Clinical research unit under Dr. V. R. Khanoikar at the Indian Cancer Research Centre, Bombay.

1. A study on the non protein nitrogen (NPN) constituents of serum and urine in healthy subjects and in cases of liver and kidney dysfunction was continued and the following normal and pathological cases were studied:

Cases	*Number*
Healthy subjects ...	31
Cirrhosis of liver ..	6
Cancer with secondary liver dysfunction ..	6
Cancer with secondary kidney dysfunction ..	7

The following conclusions have been drawn :

(i) The urinary excretion of ammonia N in liver cirrhosis cases is high in comparison to that of healthy subjects, while the excretion of NPN, Urea N, Uric acid and Creatinine is lower than that in healthy subjects. This finding is in agreement with that of Pain and Banerjee (*Indian J. M. Res.* 45 : 35, 1957) who have studied urinary excretion of NPN constituents in cases of cirrhosis of liver.

(ii) Liver or kidney dysfunction cases, complicated with cancer, do not show significant changes in the serum and urinary levels of NPN constituents.

2. Estimation of Gonadotropic Hormone (GTH) Levels in urine by a chemical method : The chemical method for the estimation of GTH depends upon the selective adsorption of the hormone on $Al(OH)_3$. Since phenol, tyrosine and albumin give the same colour reaction with Folin-Ciocalteu reagent as gonadotropins, a study of the specificity of adsorption of GTH on $Al(OH)_3$ was considered essential. Control experiments, therefore, were carried out with addition of phenol, tyrosine and albumin to urine samples and it was found that these substances

were not adsorbed on $Al(OH)_3$ as did the gonadotropins, but remained in solution. Having established this fact, estimations of the GTH in male and female urines were carried out by the method described in last year's report. Results of the work are summarised below :

Material	*No. of subjects*	*GTH**	
		Range	Mean ± S.E.
Normal males	33	2.78 —18.5	8.19 ± 0.70
Normal females	6	5.20 — 9.30	7.46 ± 046

*Expressed as mg. of casein in 24—hour urine sample.

Since morning samples of urine in the case of pregnancy and vesicular mole are known to contain high percentage of GTH, four samples from both these categories were estimated by the method mentioned earlier and, as expected, high values for GTH were obtained. In cases of testicular tumour (6), vesicular mole (4) and pregnancy (9) also a high titer of GTH, was obtained.

Bioassays of GTH on immature mice (19–21 days old) using estrone as reference standard (as suggested by Rosenberg et al., Endocrinology, 16: 337, 1957) were also carried out. The values obtained by the method were in conformity with those obtained by the chemical method.

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2. Neurological unit under Dr. V. R. Khanolkar at the Indian Cancer Reserch Centre, Bombay.

I. Pathology of Kyasanur Forest Disease.

(a) During the year, material from one human autopsy, and from 35 monkeys collected from the forest areas in Mysore and 18 monkeys inoculated in the laboratories of the Virus Research Centre, Poona, was received for study. The pathological findings in the brains and viscera were essentially similar to those encountered in the specimens received during 1956-57, and were consistent with the view that Kyasanur Forest Disease is a viraemia with haemorrhagic manifestations.

(b) Histological examination of brains and other tissues of a total of 55 cases of suspected encephalitis received from Singapore were completed. The large majority of the brains revealed changes consistent with those seen in arthropod-borne virus encephalitis cases. There was in addition material from three cases of an acute illness termed "Gurkha Infant Disease" the most striking feature of which was an unusual elevation of the protein content of the C.S.F. The pathological findings in this material are being prepared for publication.

(c) In addition to the specimens mentioned under items (a) and (b) 122 other specimens of neuropathological interest were received by this Unit for study. Included among these are the brain spinal cord and viscera of a case of suspected lathyrism and specimens from four horses suspected to be suffering from equine encephalitis.

II. Studies in Lathyrism

Experimental observations on a modified approach to the production of lathyrism in animals, referred to in previous reports are being concluded. The results obtained so far do not support the hypothesis on which this modified approach was based.

III. Observations on Pyridoxine Deficiency

Biochemical observations on blood and liver tissue of rats with induced pyridoxine deficiency (both dietetic and with the use of desoxy-pyridoxine) and on one monkey with a pure dietetic deficiency have been concluded. Histological examination of the tissue of these animals is in progress.

IV. Neurophysiology Section

As a preliminary step to the development of this section, 12 full electronic instruments have been assembled and tested.

3. Clinical research unit under Dr. R. N. Chaudhuri at the School of Tropical Medicine, Calcutta.

A. *Amoebiasis :*

Experimental study.—Studies were made with the aid of a small apparatus, which can be permanently fixed into the caecum of experimental animals (guineapigs) as reported last year, and through which a crude culture of *E. histolytica* can be introduced to cause infection of the intestine or caecal contents withdrawn to observe the behaviour of the amoebae (e.g. under the influence of drugs). It permits a variety of observations without sacrificing animal. With the fixture *in situ* the animals can thrive almost normally for an indefinite period and the tube if cleansed from time to time, maintains its patency.

Such animals when inoculated with 48 hours' crude culture of *E. histolytica* directly into the caecum through the tube usually acquire the infection within a period of 8 to 10 days. In a few instances, the animals had to be inoculated for the second or even third time, for successful infection. The trophozoites gradually increase in number as revealed by daily examination of caecal contents. The animals become sick with disinclination for food and lose considerable weight but without any diarrhoeic manifestation. They eventually die in about 4 to 8 days after infection. Autopsy reveals typical ulcers in the caecum, the scraping from the wall showing numerous trophic forms of parasites.

With this preliminary observation, it was considered worthwhile to explore the possibility of utilising this method for screening of amoebicidal drugs. Altogether five different amoebicidal drugs were tried and the progress during treatment was assessed by daily examination of caecal contents for *E.histolytica*. The animals that responded to the drug were followed up for relapses with bi-weekly examination of caecal contents. The results obtained with different drugs are as follows :

(*a*) *Emetine-Bismuth Iodide* was tried in a group of four infected guineapigs in a daily dose of 1 mg. for 10 days. Two animals responded on the 4th and 6th day of treatment and during 2 weeks' follow-up period there was no relapse, while in the remaining two it proved ineffective. No toxicity was observed.

(*b*) *Carbarsone* in a daily dose of 25 mg. was given to four infected animals and all of them responded on the 2nd and 3rd days, of treatment. But the drug being very toxic was discontinued on the 4th day. No relapse was observed during two weeks' follow-up.

(*c*) *Kurchi-Bismuth Iodide* was tried in four infected animals in daily doses of 128 mg. for 10 days. The caecal contents became negative of *E.histolytica* on the second and fifth days of treatment in 2 of them and both had a relapse after 17 and 38 days, respectively. In the other two animals the drug was ineffective. There were no toxic reactions.

(*d*) *Ipecac-Bismuth Iodide complex* was administered to a group of eight guineapigs in a daily dose of 6 mg. for 10 days. Three animals responded on 3rd, 6th and 8th days of treatment respectively, of whom

one died apparently of drug toxicity on the 9th day of treatment, one relapsed after five weeks and the remaining one remained well during three weeks' follow-up. Toxic reactions were observed in four animals.

(e) *Mebinol V* (ethoxy-ethyl-phenoxy-nitro-benzyl-dichlor-acetamide) was tried in eight guineapigs in a dose of 120 mg. daily for 10 days. Six animals responded within 2—4 days of treatment all of whom relapsed between 9 and 35 days after the completion of treatment. In the remaining two the infection was uninfluenced. No toxicity was observed.

Without commenting on the efficacy of the drugs from the limited observation it appears that the above technique is suitable for the study of experimental amoebiasis not only in determining the onset and course of amoebic infection but also in screening of chemotherapeutic agents. It may as well facilitate recognition of relapses and resistance of the organisms to drugs and influence of diet, bacteria and other measures on the infection.

B Tropical Eosinophilia :

The unit undertook (i) studies on the role of parasitic infection in its aetiology and (ii) clinical studies.

(*i*) *Experimental study.*—Eosinophilic response in animals fed with ascaris larvae—Twenty-four rats were fed with a suspension of embryonated eggs of ascaris lumbricoides (supplied by the Helminthology Deptt. of the School) in normal saline having 1000—3000 eggs per feed. The blood was examined before the experiment and thereafter on alternate days or twice a week throughout the experimental period. The rats were divided into two groups of twelve each according to the regime of feeding, as shown below :—

Group 1 : Six feeds were given at an interval of 3 weeks. There was a rise of eosinophil count in eight of them from the initial level 0-2 per cent (absolute eosinophil count 0-150/c.mm) to a maximum of 10-22 per cent on the 9th to 13th days after the first feed. Such a rise, however, came down to normal spontaneously within the next 3-6 days. During the period following the second and third inoculations there was still higher rise of eosinophils though temporary up to 28 per cent (absolute count 2300/c.mm). With subsequent feeding the response was, however, very poor.

Group 2 : To ascertain if this process of transitory increase of eosinophils may be perpetuated the other twelve rats were fed with the same dose daily for 4 weeks. The resulting eosinophil percentage up to a maximum of 30 per cent (absolute count 3700/c.mm) persisted for a longer period (one to three weeks) but came down spontaneously although the feeding was continued.

In eight control rats fed similarly with normal saline the percentage of eosinophils varied between 0 and 3.

In another set-up sixteen guineapigs were fed with 300 to 500 embryonated eggs suspended in normal saline daily for 6 weeks. The range of initial eosinophil count was between 0 and 4 per cent. All the animals showed increase of eosinophils in peripheral blood after 9 and 13 days of experiment and the maximum rise of 20 to 33 per cent was attained on 14th to 32nd days. Thereafter a gradual fall was noticed inspite of regular feeding being continued. The animals were again fed with a single dose of eggs (3000) showing little or no eosinophilic response.

Skiagram of lungs taken at the height of eosinophilia in six animals did not show any significant change.

The histological examination of lungs of these animals showed marked eosinophilic infiltrations with fragments of ascaris larvae in some areas.

It appears that ingestion of embryonated ascaris eggs causes eosinophilia with eosinophilic infiltration of lungs. The eosinophil cells increase in number if the dose is repeated two to three times. But in any case, the effect cannot be perpetuated by subsequent feedings even with high concentrations of larvae. This simulates the syndrome described in 1932 by Loeffler, who held that it was an allergic response to migration of ascaris larvae in the lung. It is possible that with repeated re-infection some immunological reactions develop in the host which prevent eosinophilic response to occur indefinitely.

(ii) *Clinical study.*—Diethyl carbamazine tried in 90 cases of tropical eosinophilia in doses of 8-12 (50 mg. each) daily for varying periods. The results were usually good, but seven patients failed to respond. In most cases, the pulmonary symptoms and signs disappeared within a week and the blood count reached the normal level in 2—5 weeks on an average. Its toxicity was low. Seven out of thirty-two cases followed up for a period of 1—2½ years had a relapse which responded well to the same drug again. None of the thirty cases followed up for one year or less had recurrence.

C. *Liver in diabetes* :

Following detention of diabetes in a few cases of chronic obscure splenomegaly, the Unit undertook studies of liver to find out its correlation if any, with the degree, duration and/or treatment, diet, etc., of diabetes.

(i) *Experimental study.*—To find out the significance of liver involvement in the course of alloxan diabetes some experiments were carried out in 3 groups of rats, one of which was control. In the other two groups the liver was damaged by carbon tetrachloride injections or by a synthetic diet for six weeks which produced fatty changes or cytoplasmic degeneration of the liver cells.

It was observed that—(i) If the animals (normal) are starved for 24 hours and then given a single injection of alloxan in a dose of 20 mg. per 100 g. of body weight, hyperglycaemia and glycosuria are produced, in most of them in 2-3 days. (ii) If the liver is damaged

in the way described above the diabetic condition appears much earlier.

(ii) *Clinical study.*—Eight juvenile cases of diabetes, aged 12 to 22 years, were investigated as to the state of their liver after admission. Duration of diabetes was 1 to 7 years. None except the patient with 7 years duration had any dietetic or insulin treatment. Liver was palpable in six. Total serum protein, albumin/globulin ratio and thymol turbidity values did not show any significant alteration except in 2 cases of 4 and 7 years duration. Serum bilirubin was normal in all. Liver biopsies showed no significant change in histology except moderate fatty change in 2 cases and nuclear vacuolation in three.

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4. Schistosomiasis enquiry under Dr. R. K. Gadgil at the Grant Medical College, Bombay.

Infection of Ferrissia tenuis.—During this period attempts were mainly directed for obtaining an adult schistosome from the infected animals in the laboratory. With this aim in view, following experiments were carried out. During the various periods small and large batches of Ferrissia tenuis snails were infected with the miracidia of S. haematobium. Certain variations were made with the methods of infection in each batch. It is our experience that whatever methods we follow, the high mortality rate in the infected snails could not be prevented. It had been possible to infect the snails but the number of cercariae obtained was very small. The number of snails that survived the period was also considerably low. These difficulties created a major problem in obtaining large number of cercariae for the infection of animals in the laboratory as well as at the village Gimvi. However, with the small number of cercarie available, animal infection experiments were also carried out.

On re-assessing the difficulties in these infection experiments it was decided to infect the animals in the area of the endemic focus. Small pools of water were prepared in the rivulet itself, and snails were infected in these pools under natural conditions. The infection of these snails was possible because of the co-operation of the village school-teacher, who directed the pupils to pass urine at these selected spots. Snails were collected from these spots after a month, and animal infections were carried out. It was noted that the cercarial yield was very low in this case. It is proposed that this would be the method of choice to be followed for obtaining an adult worm.

Infection of animals.—In the month of March, April, 1958, 12 mice were infected at Gimvi with the cercariae emitted by the naturally infected snails. Three such infections were given. All these were found to be negative after a period of 3 months. In May, 1958, similar infections were given to 18 mice at Gimvi. These animals also failed to take up the infection.

In the laboratory 10 mice were infected in the aquaria containing the infected *F. tenuis*. Three such infections were given. These also proved to be negative.

Six mice were infected with the cercariae emitted by the Indo-planorbis exhustus snails. Out of these only one mouse showed the presence of 6 adult worms of Schistosoma spindale.

Viability of ova.—Experiments on the viability of ova were carried out with the medium of different types of soils. It was observed that in the soil consisting of fine gravels, moistened with water, ova were viable up to 48 hours.

Examination of stool samples.—Along with this work certain other investigations were undertaken. In one previous report, a case of schistosomiasis from Mudh-Island was reported. The ova were detected in stools. Similar cases were noted by Dhanda, (1956) in Delhi. It was

decided to examine consecutive samples of stools from the O.P.D. of the J. J. Group of Hospitals. The stools were examined by different concentration methods including Merthiolate Iodine Formaldehyde Concentration technique suggested by Blagg and others (1955). It was found that the results obtained by the MIFC method were more satisfactory in comparision with the other methods. The incidence of *E. histolytica* was 25.12 per cent, *Giardia* was 14.39 per cent and *Ascaris* was 10.37 per cent.

A similar type of work was undertaken later. 274 stool samples were examined from the village Depoli of Palgarh taluka. Incidence of round worm was as high as 73.4 per cent. Whip worm was 59.7 per cent *E. coli* was 28.9 per cent and *E. histolytica* was 18.6 per cent Schistosome ova were not detected in any of the samples.

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5. Inquiry into the use of artificial hypothermia (Hibernation) in open intracardiac surgery under Dr. P. K. Sen at the Seth G. S. Medical College, Bombay.

Altogether sixty-five experiments have been carried out during the year. Work was done under two main heads :—

A. *Studies in extreme hypothermia*—to carry out open 'dry' cardiotomy on completely non-pulsatile 'standstill' heart with a view to exclude cerebral anoxia, ventricular fibrillation and air embolism. A group of eighteen experiments to study the behaviour of the heart at low temperatures ranging from 23°C to 10°C, have been carried out.

B. *Studies in elective cardiac arrest*— for open cardiac surgery, with cardioplegic drugs combined with, or without hypothermia. This work has been done in the following six phases :—

1. Use of potassium citrate alone.
2. Use of acetylcholine alone.
3. Combination of potassium citrate in different concentrations with acetyl-choline, at normal body temperature.
4. Combination of potassium citrate with acetyl-choline under hypothermia.
5. Combination of potassium citrate with acetyl-choline under hypothermia, using 10per cent sodium lactate to wash out the cardioplegic drug from the coronary circuit.
6. Combination of potassium chloride with acetyl-choline under hypothermia.

(A) Experimental Procedure

Adult mongrel dogs were anaesthetised with intravenous sodium pentothal (30 mg. per Kgm. body weight); an endotracheal tube was inserted and the lungs ventilated at the rate of 48 times per minute by a positive pressure respiratory pump. Venesection was done and an intravenous drip of 5 per cent glucose started. Electrocardiographic control tracings were taken and animals immersed in ice cold water (0° to 4°C temperature) after recording the original temperature with a rectal electric thermo-couple. The rectal temperature and the E.C.G. records were maintained every ten minutes. When the required low temperatures were obtained cold water was siphoned off and replaced by tap water. The temperature of the bath was raised to 45°C by addition of warm water and the animals re-warmed to their original temperature.

Out of 18 dogs, thirteen went into complete cardiac arrest. Five of these did not recover on re-warming while eight were completely revived. The remaining five dogs showed a heart rate of 4 to 8 per minute at the lowest temperature of the experiment. Of these, one died during re-warming, two died 4 hours after they had been restored to their original temperature while two survived.

The cooling time during all these experiments varied from 1 hour 30 minutes to 5 hours 50 minutes. The period of arrest in the animals that were revived varied from 8 to 25 minutes.

In our previous work on extreme hypothermia a series of 18 dogs were cooled to temperature 5 between 9°C to 15°C. Complete cardiac arrest was obtained in 7 animals of which only 3 could be revived back, the other four did not register any heart-beat on re-warming. In the remaining 11 animals, heart rates from 4 to 6 per minute were obtained at the lowest temperatures of the experiment. All of these were revived on re-warming. Cooling time for arrest or near-arrest stage varied from 1 hour 25 minutes to 3 hours 10 minutes. Period of arrest in the animals that were revived varied from 20-45 minutes.

(B) Experimental Procedure

Adult healthy mongrel dogs weighing between 10 and 15 Kgm. were used. Anaesthesia was induced by intravenous sodium pentothal (30 mg. per Kgm. body weight). No predication was employed. Animals were kept on positive pressure ventillation with air by a respiratory pump through an endotracheal tube at the rate of 20 per minute except when hypothermia was used where the rate was kept at 48 per minute.

The chest was opened transversely through the 4th space; the sternum divided and both the internal mammary arteries ligated. The azygos vein was ligated. Superior and inferior venae cavae were dissected and loops of silk passed round them. The pericardium was dissected and lifted off the roots of the great vessels. The pre-aortic pad of fat was excised and the root of the ascending aorta dissected free. A loop of silk was then placed around the aortic root.

The superior and inferior venae cavae were occluded by bull-dog clamps (this inflow occlusion was practised in all cases before bringing about cardiac arrest so as to prevent distension of the heart by the returning venous blood). The ascending aorta was clamped about one inch away from the aortic valve and the cardioplegic drug injected by a No. 18 B.D. needle until the heart stopped in diastole. After an interval of time depending on the type of arrest and varying from a fraction of a minute to several minutes attempts were made to wash off the cardioplegic drug from the coronary circuit. The solutions used for this purpose were either whole blood, Ringer's solution, 5 per cen glucose, normal saline or 10 per cent sodium-lactate. As soon as a definite heart beat was established the outflow and inflow clamps were removed and artificial ventilation re-started. In some instances injections of calcium, adrenalin, or neostigmine aided by cardiac massage were used to bring about restitution of the heart to normal rhythm. The chest was then closed in layers without any drainage.

Throughout the experiment continuous E. C. G. readings were recorded. Standard limb leads and unipolar augmented limb leads were employed, lead II being the one most frequently employed.

Cooling of the dogs in cases where hypothermia was used and

brought about as per routine described in our procedure (A) above. The animals were taken to only a moderate degree of hypothermia i· these series -- around 30°C to 32°C temperature.

The observations as made in the various phases are as follows :—

1. *Use of potassium citrate alone.*—In all 10 experiments were done. Potassium citrate solution 5 per cent to 25 per cent was used till the heart stopped. The period of diastolic arrest was from 1 to 4 minutes. The amount of potassium citrate used varied from 400 to 1200 mg. During resuscitation all the dogs went into ventricular fibrillation. In spite of all resuscitative measures such as massage, injections of calcium chloride, adrenalin and neostigmine none of the dogs could be revived. The temperature used varied from 30°C to 32°C. One dog died as a result of the endotracheal tube accidentally slipping out during the experiment.

2. *Use of acetyl-choline alone.*—Nine dogs were treated with Acetylcholine 1.5 per cent solution 10 mg. per kgm. body weight In all cases a diastolic arrest not exceeding 1 minute was obtained. Eight dogs were restored to normal rhythm after washing the coronary circuit with blood. One dog went into ventricular fibrillation and could not be revived. Two of the revived animals were sacrificed on the table for other purposes; five dogs died after twenty-four hours and one after eight days. There was no evidence of brain damage in any of the animals. The experiments were conducted at 37°C temperauture.

3. *Combination of potassium citrate* (*1* per cent) *in different concentrations with acetyl-choline* (*1.5* per cent).—Five experiments were conducted with these drugs at 37°C temperature. Complete diastolic arrest varying in duration from 2 to 5 minutes was obtained in all cases. Four dogs were revived in spite of going into ventricular fibrillation while in one dog ventricular fibrillation did not revert to normal rhythm. Restitution time varied from 4 to 20 minutes. All dogs died subsequently due to brain damage.

4. *Combination of potassium citrate with acetyl-choline as in 3 but under moderate hypothermia.*—Thirteen dogs were subjected to this procedure. Dogs were cooled to a temperature of 30°C to 32°C. All went into a complete diastolic arrest of 2 to 7 minutes duration. Potassium citrate 7 to 10 mg. per kgm. body weight and acetyl-choline 10 mg. per kgm. body weight were used. During revival all dogs went into ventricular fibrillation. Eleven dogs could be revived by various restitution measures. Two dogs did not recover. The revival time varied from 1 to 20 minutes. Evidence of brain damage was definite in two animals, doubtful in two others and absent in the rest. The survival time varied from 1 to 3 days.

5. *Combination of potassium citrate with acetyl-choline under hypothermia as in No. 4, using 10*per cent *sodium lactate as the*

washing solution instead of blood.—Five experiments were done. Diastolic arrest of 3 minutes was obtained in all cases. Two were revived within one minute without going into ventricular fibrillation, while three animals died due to ventricular fibrillation.

6. *Combination of potassium chloride 1.2* per cent *with acetylcholine 2* per cent *under hypothermia using 10* per cent *sodium lactate as washing solution.*—Five experiments have been done so far. Diastolic arrest ranging from 3 to 5 minutes, has been obtained in all five. Of these, three recovered without going into ventricular fibrillation within 10 minutes, the other two went into ventricula fibrillation and could not be revived.

6. Investigation into the role of allergens and various other factors in the production of bronchial asthma in Rajasthan in general, and Jaipur area in particular, under Dr. R.M. Kasliwal at the S. M. S. Medical College, Jaipur.

The work of the enquiry was planned for preparing a pollination calendar and investigating the importance of other well known inhalant allergens of this area. The pollination calendar is a record of the allergenic plants and of the time (in days and months) when they effloresce and liberate enough pollens in the atmosphere to cause respiratory allergies. Other inhalants investigated were such well known allergens as house-dust, fungi, animal dander, wool, cotton, etc. The work was planned as under :—

1. Botanical survey of the area, to identify the local trees, weeds and grasses.

2. Collection of plant pollens at the time of their efflorescence and preparation of their extracts suitable for testing.

3. Determination of allergenic pollens by performing skin tests with their extracts.

4. Study of atmospheric pollens and their correlation with the clinical history of the patients and the positive skin reactions.

5. Preparation of a pollination calendar.

6. Determination of the allergenic nature of other inhalants already mentioned.

Nos. 1 and 2 of the above work were reported in the previous years 1955-56 and 1956-57. Technics of testing and interpretation of test were also reported earlier. All the plants of this region were collected fo botanical identification. 70 trees, 19 weeds and 45 grasses were thus investigated. On perusal of botanic details it was realised that man of the plants were unimportant from the point of pollination calenday Only the anemophilous and these entomophilous plants which grow densely or shed light pollens capable of floating in the air were considew ed. Thus 30 trees, 7 weeds and 18 grasses which fulfilled these criterir were extracted for investigation of their allergenic role. The allergen city was determined by noticing the skin reactions in cases of prove respiratory allergies. Only those reactions which could also be tallied with the history at the time of pollination of the incriminated plants were considered significant. Actually it was observed that significant reactions were given by only those pollens which were allergenic. 100 patients of asthma, 22 of hay-fever and 38 of allergic rhinitis were investigated. Of asthma 29 were perennial, 40 perennial with seasonal exacerbation and 31 were seasonal. The plants that gave significant and non-significant reactions are shown below :

ype of lant.	*Plants giving Significant reactions*	*Plants giving non-significant Reactions*
REES	Ailanthus, Dodonaea, Holoptelia, Morus, Parkinsonia, Phoenix, Phyllanthus, Pithecolobium, Prosopis & Putranjiva.	Acacia arabica & farnesiana, Aegle, Albizzia, Caesalpinia, Callistemon, Cassia, Cuminum Delonix, Enterolobium, Eucalyptus, Ixora, Leucaena, Lawsonia, Melia, Moringa, Nicotiana, Psidium, Raphanus and Salix.
'EEDS	Amarantus gangeticus and spinosus, Argemone, Chenopodium, Elatostema and Ricinus-	Datura
GRASSES	Chloris, Cynodon, Dactyloctenium, Desmotachya, Digitaria, Elusine, Pennisetum typhoideum, Saccharum, Sorghum halepense & Zea-mays.	Arundo, Cenchrus, Eragrostis minor & tremula, Panicum, Pennisetum dichotomum, Polypogon & Sorghum vulgare.

After having determined the significant allergenic plants of the area, the investigation was extended to determine which of the pollens actually float in the air. For this purpose a catchment slide was placed on the highest point (roof) of S.M.S. Hospital and pollens caught on it were studied. The names of the plants whose pollens were thus caught are given below —

TREES	Ailanthus, Dodonaea, Holoptelia, Morus alba, Phoenix, Phyllanthus, Prosopis, Putranjiva and Salvadora.
WEEDS	Amarantus-Chenopodium & Elastostema group, Cannabis, Cyperus, Ricinus.
GRASSES	The grass pollens cannot be differentiated from each other.

On comparing pollenes found allergenic on testing and pollen caught on the slides, it was found that all important allergenic pollens were caught on the slides except Parkinsonia, Pithecolobium and Argemones. The grasses Dactylctenium, Digitaria and Elusine, though found allergenic on testing, produce meagre qnantity of pollens and thus are not of much clinical importance.

From the point of view of pollination calendar the plants whose pollens were caught on the slide are really important. Other pollens which are allergenic but do not attain any concentration in the air will only cause allergy if inhaled directly. This group will also include many ornamental plants like rose, mary-gold etc. which do not constitute part of the present investigation. The pollination calendar of common and important plants, is given below.

Name of plant	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.
Phoenix	xx	xx	x	x	x	x						
Prosopis	x	xxx	x									
Morus			x	x	x	xxx	xx					
Ailanthus				x	xxx	xxx	x					
Salvadora				x	xxx	x						
Dodonaea					x	xxx	x					
Holoptelia						xx	xxx	xx				
Putranjiva							x	x	x			
Phyllanthus							x	x	x			
Am-Che-El group	xx	xxx	xxx	xxx	xxx	xx	x	x				x
Cyperus	xx	xx	x								x	x
Ricinus	x	x	x									x
Cannabis					xxx	xxx	xxx	xx	x	x	x	
Grasses	xxx	xxx	xxx	xx	x						xx	xxx

(x indicates aerial pollen concentration of less than 50, xx between 50-100 and xxx more than 100 per sq. cm. of the slide exposed, in monthwise distribution).

A few general conclusions may be drawn from the above table. The trees excepting Phoenix and Prosopis pollinate in and after January. Phoneix is apt to cause symptoms starting from September and lasting through February while Prosopis starts in September and ends in November. Grasses on the contrary, are apt to cause maximum symptoms during rainy season and autumn. Of the weeds, the Amarantus-Chenopodium Elastostema group begins to appear in the air from August but attains maximum concentration only during November. It is noteworthy that when this group achieves the highest concentration in air the grass pollens show a decline which fact will help in differentiating the weed allergy from grass allergy. The pollinating periods of Cyperus and Ricinus, however, coincide with those of grasses and have to be clinically differentiated and appraised. Pollens of Cannabis are most abundant in and after January and they last till after April when their concentration repidly declines.

On studying the seasonal frequency of individual pollens it could be said that grass allergy is clinically significant during rainy season and autumn, weeds during autumn and early winter and trees during late inter and spring.

Of the other inhalant allergens, house-dust was found to be the commonest offender. It was responsible for allergic manifestations in 73 per cent of cases; either alone or in combination with other allergens. This high incidence, though reported in other countries as well, stresses the importance of house-dust in Indian evironment. Of the fungi, rusts, smuts and molds of the area were investigated. The rusts and smuts which were obtained on fiield survey were extracted and tested. Of all the patients tested only 10 gave grade I positive reaction. No significance could, however, be attached to these reactions as on further analysis of history of the disease and occupation in these patients, the reactions were found to be non-significant.

Molds cannot be obtained in pure form in nature and hence are being cultured in the laboratory. Their allergenic character is well known. The catchment slides set up in the area have been showing a high concentration of molds in the air. It is hoped that in a few months, their importance in this region will be fully investigated.

One case was found to be sensitive to wool, 4 to cotton-seed and 10 to animal danders. In the latter group 5 were sensitive to dog hair and 5 to camel hair.

The present research has been of clinical importance since it provides : (i) a pollination calendar for this region enabling the investigation and diagnosis of the cases of seasonal allergy. (ii) enables institution of specific treatment, based on the discovery of the causative allergens, (iii) and besides, it establishes the role of other respiratory allergens that are common in this region.

7. Enquiry on the value of commercial silk grafts to bridge large blood vessel gaps under Dr. Yudhveer Sachdev at the Medical College, Amritsar.

In the year 1956 and 1957 homografts preserved in 4 per cent neutral formalin solution at room temperature, 70 per cent Ethyl alcohol, balanced salt solution containing 10 per cent homologous serum and antimicrobial drugs at a temperature 2°C to 6°C were implanted in the abdominal aorta of 30 dogs

Material and Method

Silk tubes were prepared from a variety of Kashmir silk known as Tabi by sewing with sewing machine. Three types of silk tubes (double layer, single layer untreated and single layer treated) were used to bridge the gap in the abdominal aorta of the dogs below the renal arteries. Before use these tubes were finely trimmed, mounted on glass rods and sterilized either by autoclaving or boiling. The tubes were treated with blood and then fixed in alcohol. In the later series 500 c.c. 1000c.c. of 5 per cent glucose was also given by arapid drip at the end of the operation combat to shock. In 10 dogs penicillin was sprayed locally and also given par enterally in the post-operative period. Retrograde femoral ao rtography was done in 6 dogs at variable periods after the operation to visualise the condition of the prostheses. Macro and microscopic study of the prostheses removed was done in detail.

Results

Silk prostheses were impladted in the abdominal aorta of 48 dogs, with following results :—

Seven dogs are still under observation with palpable femoral pulsation 216, 144, 125, 102, 83, 83 and 4 days after the operation. Retrograde femoral aortography, done in 6 of them, revealed patent prostheses.

In 8 dogs prostheses were found to be patent and suture lines normal. Femoral pulsation remained palpable in all of them till the last day. Out of these 4 were sacrificed 17, 40, 42 and 67 days after the operation. One dog was killed during fight with another dog 2 days after the operation and 2 died of haemorrhage, 13 and 15 days after the operation. In one of these the source of haemorrhage could not be traced and in the other it was due to opening of lateral seam and since then double seam is applied as a routine. One died of acute dilatation of the stomach on the 2nd post-operative day.

In 9 dogs prostheses remained patent for 2, 2, 5, 12, 19, 20, 31, 51 and 64 days after the operation, as observed clinically by palpable femoral pulsation and later thrombosis occurred.

In 6 dogs, femoral pulsation remained palpable for 4, 6, 12, 14, 37 and 45 days after the operation when they suddenly died of haemorrhage due to partial dehiscence of suture line. Prostheses in these dogs were found to be patent.

In 4 dogs partial dehiscence with thrombosis occurred 4, 20, 23 and 69 days after the operation.

Thrombosis with paraplegia within 24 hours occurred in 8 dogs.

In 4 dogs postmortem could not be done. In 2 femoral pulsation disappeared within 24 hours of the operation and in the other two remained palpable till the last day when they died 4 and 12 days after the operation.

Two dogs absconded and femoral pulsation in these remained patent for 18 and 21 days.

Gross Pathology :

Out of the 48 dogs the prostheses were examined in 35 dogs. Fibrous tube was formed around 19 prostheses. This tube was formed during the second week and was loosely adherent except near the suture lines. Luminal surface of the prostheses was covered by fibrin layer in 12 dogs. It was about 1 mm. thick and resembled intima of the host aorta in prostheses removed 6-7 weeks after the operation. There was no inner lining in prostheses in which early or late thrombosis occurred. It was loosely adherent except near the suture lines.

Histopathology :

Histopathological examination was done in 28 prostheses which revealed that the inner fibrin lining was gradually replaced by fibrous tissue starting near the suture lines. Outer tube contained fibrous tissue, chronic inflammatory cells and foreign body gaint cells. No fibrous tissue was found growing through the interstices.

8. Study of renal changes following ureteric ligation and an assessment of recovery following release of obstruction by ureteric transplantation under Dr. B. N. Balkrishna Rao at the G. R. Medical College, Gwalior.

From the work conducted on this enquiry in the last three years, the following conclusions have been drawn :—

(i) Sudden complete obstruction to the ureter invariably produces hydronephrosis and not primary renal atrophy as is usually believed.

(ii) By study of retrograde pyelograms, dye excretion tests (Indigocarmine and P. S. P. tests), blood urea and histopathology, it has been concluded that critical time period for reversal of hydronephrotic changes is 21 days.

(iii) The higher the obstruction in the ureter the quicker hydronephrosis develops and more the renal damage.

(iv) Incomplete obstruction to the ureter also invariably produces hydronephrosis. This hydronephrosis is much slower to develop and produces lesser damage to renal tissue then that of hydronephrosis by complete obstruction.

The aim of the present scheme is to elucidate the effects of gradual, incomplete obstruction, to determine the etiology of the so called idiopathic hydronephrosis and to investigate certain other factors concerned with experimental hydronephrosis, for example, the study of the rate of growth of stone in hydronephrosis sac or urinary bladder and the study of changes in lymphatic pattern.

The changes in back pressure irrespective of the nature of primary obstruction are of importance due to the ultimate effect they have on the kidney. Discussion on this subject has long interested physiologists, pathologists, as well as surgeons and urologists. The results of the present work differ fundamentally from previously published reports, especially on restitution to normal of hydronephrotic kidney. In the present work, contralateral nephrectomy carried out to exclude the effect of renal counterbalance still led to functional recovery.

This was conclusive evidence of the recovery of the kidney which was formerly hydronephrotic.

The knowledge gained through experimental work so far, has the following important therapeutic applications :—

(i) Conservative treatment of ureteral obstructions may be followed without any increased risk for a much longer period than is at present practised.

(ii) Nephrectomy need not be carried out so frequently in cases of obstructive hydronephrosis. It should only be performed if, after removal of obstruction to allow the renal parenchyma optimun facilities for recovery, there has not been the expected progress.

9. Inquiry into experimental production of pneumoconiosis and emphysema under Dr. R. K. Goyal at the S. M.S. Medical College, Jaipur.

The work reported till the end of September 1957 had shown that monkeys and dogs are very susceptible to pneumoconiosis, sheep and goats are less susceptible, whereas rats, rabbits and guinea-pigs are rather resistant. The susceptibility of pigs and cats was next investigated. Five pigs and fifteen cats in all were examined for this purpose.

The results indicated that cats and pigs are not highly susceptible to pneumoconiosis. The presence of co-existing infection appears to be necessary for the production of early pneumoconiosis in these animals.

In the present series of experiments the effects of added infection were investigated. As many as 72 rats were exposed to a mixture of dust (3 parts) and wood-charcoal (1 part). This mixture is preferred to simulate the condition to which the urban populations are usually exposed. Since the organisms isolated from the lungs of rats suffering from acute bronchiolitis or interstitial pneumonitis were usually *B. coli* (and only occasionally staphylococci), cultures of the former orgasisms only were used in infecting the animals in this experiment. Suitable controls were also maintained. The results are given below.

The rats were made to inhale dust and charcoal for periods varying from 23 to 52 days; sub-lethal doses of *B. coli* (200-300 million) were injected intraperitoneally either (a) about one month before the inhalation or (b) during the period of inhalation. The injection of *B. coli* one month before the inhalation, did not have any effect on the lesions. The lesions of early pneumoconiosis with presence of coal particles and scanty silica particles were less marked in those rats who had received *B. coli* injection during the course of inhalation *B. coli* injection produced acute interstitial pneumonitis which probably helped in the mobilization of the few carbon or silica particles already present in the lung tissue. The intraperitoneal injection of *B. coli* or a mixture of *B. coli* and Staphylococci led to the appearance of extensive peribronchial foci of lymphocytic infiltrations. These lesions resembled those produced in rats exposed to inhalation of dust, hence the lesions in the lungs of rats may be considered to be mostly non-specific. A few silica particles along with the granulomations lesions were encountered only when a chronic inflammatory lesion co-existed. These chronic inflammatory lesions could not be reproduced by injection of *B. coli* or staphylococci. It was, therefare, considered desirable to use an avian strain of tubercle bacilli to produce chronic inflammatory lesions; such a virulent strain however, could not be easily obtained. A suspension of rat leproma bacillus was obtained instead from the School of Tropical Medicine, Calcutta and the effects of its injection were investigated.

The rat leproma suspension was injected into the lung of four rats. In a preliminary experiment, a rat was exposed to dust and charcoal inhalation for 28 days after about four weeks of the intrapulmonary injection of the suspension, small amounts of silica along with carbon particles were discernible in a few small foci. It would thus appear that chronic inflammatory lesions produce chronic lymphangitis, leading to the retention of carbon and silica particles.

The above-mentioned experiments were repeated with certain modifications/using guinea-pigs. The inhalation of a mixture of dust and charcoal carried out for 16 to 38 days produced foci of lymphocytic infiltration as well as marked narrowing of some arterioles; coal and silica particles were encountered in moderate amounts when there was co-existing subacute bronchiolitis. Attempts to produce chronic bronchiolitis and lymphangitis with the B. C. G. strain of bacillus proved unsuccessful.

In the next series of experiments, 100 tubercle bacilli (virulent human strain) were injected intraperitoneally into guinea-pigs, which were then made to inhale dust and carbon mixture at varying intervals. The guinea-pigs, which developed early tubercular lesions 4-5 weeks after infection showed the presence of silica and carbon particles in moderate amounts, whereas the uninfected controls showed presence of silica only very scantily. The infected guinea-pigs which did not develop early tubercular lesions at this stage did not show any accumulation of silica particles. It is thus clear that chronic inflammatory lesions causing probably obstruction to the lymph flow are responsible for early pneumoconiosis in guinea-pigs.

The experiments were repeated using 21 rabbits this time, of which four died prematurely.

The rabbits exposed to a mixture of dust and charcoal in the inhalation chamber for 24-38 days developed definite interstitial pneumonitis and in a few instances, the vessel walls were markedly thickened, but the silica particles were either absent or were very scanty. A number of rabbits were injected, each with 10 million tubercle bacilli by the intravenous route and were made to inhale mixture of dust and charcoal for varying periods along with uninfected controls. They were sacrificed from 14 days onwards. Silica particles were in evidence in moderate amounts in rabbits showing tubercular lesions, whereas the uninfected controls had only very scanty amounts of silica excepting in one case where the uninfected rabbit developed bronchiolitis. Thus, it again became evident that the presence of chronic inflammatory lesions is required for the production of early pneumoconiosis in rabbits.

Twenty monkeys were made to inhale a mixture of dust and charcoal for periods varying from 15 to 56 days. Three monkeys were given, in addition, sublethal doses of *B. coli* by the intravenous route, the carbon and dust particles were mobilized due to the acute infection and the lesions of pneumoconiosis were consequently less severe than in the controls. Six of the monkeys which were in apparently good health, became ill and showed definite tubercular lesions on post-mortem examination when they were made to inhale dust and carbon mixture for 13 to 20 days. It would appear that pre-existing tubercular lesions were markedly aggravated by the inhalation of dust and charcoal.

Most of the monkeys, especially the older ones showed varying degrees of thickness of some of the arteriolar walls in the lungs. The thickening was generally of the nature of medial hypertrophy with degenerative changes, the lesions of endarteritis obliterans being infrequent. The medial hypertrophy indicated the existenee of pulmonary

hypertension, which might lead eventually to cor pulmonale. No evidence of advanced heart failure was seen in the experimental animals.

Further work is required to elucidate the fact whether this benign pneumoconiosis does really cause an increased susceptibility to infective lung diseases.

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10. Inquiry on bio-microsocopic study of the conjunctival vessels in relation to the general arteriosclerosis and coronary artery disease under Dr. K.N. Mathur, Dr. K.S. Mathur and Dr. P.N. Wahi at the Medical College, Agra.

Abstract of the work done during the year 1958.—In the year 1958, 35 cases of essential hypertension and 20 cases of coronary heart disease were examined and the elasticity of the conjunctival vessels was determined by the same old technique which was perfected in the past years and in which priscol, a vasodilator drug, was used.

Fifty normal cases were also examined during this year by a new technique in which a vasoconstrictor drug like adrenaline hydrochloride was used in place of priscol for finding out the elasticity of conjunctival vessels.

Results of the Hypertensive cases examined with Priscol.—Blood vessels of diameters of .1, to .2 mm. were observed in all the hypertensive cases, while vessels of .3mm. diameter were seen in only 32 per cent cases. In the remaining 68 per cent it was not possible to demonstrate vessels of .3 mm. diameter. These later cases belonged to the older age group.

A change from the normal was also observed in the pattern of the conjunctival vessels in hypertension. Tortuesity, loops, sharp bends, irregularity of the lumen of the vessels and aneurysms were common in these cases.

Along with the changes in the pattern of the conjunctival vessels in hypertension, a diminution in their elasticity was also observed. It was seen that the conjunctival vessels did not dialate to the same extent with priscol as observed in the normal individuals of the same age group. When compared with the normals of the same age group, it was also observed that the vessels showed a delay in the onset of dilatation, and an increase, in the duration of maximum dilatation and the time taken by them to return back to their original diameter.

All cases of hypertension with the above findings in the conjunctival vessels showed sclerotic changes as well as loss in elasticity of the vessel wall.

Results with Adrenaline hydrochloride.—In spite of very satisfactory results obtained by the use of priscol, one great inconvenience was felt. The priscol test was found to be very much tiring to the patient as it took nearly $1\frac{1}{2}$ to 2 hours or even more to complete the examination. This necessitated exploration for some other drug which when used would help to shorter the duration of the test. Adrenaline hydrochloride being a short acting drug, was ultimately choosen.

To start with, 50 normal subjects were examined with different dilutions of adrenaline hydrochlorde to find out if there is a variation in the threshold in different age groups. This being not so, it is now decided to use 1: 1000 dilution of adrenative hydrochlorde for all the age groups. The investigations are in progress.

11. Inquiry on biochemical studies on tumor under Dr. S.C. Roy, Department of Applied Chemistry, Calcutta University, Calcutta.

Biochemical.—Ten different samples of blood plasma from patients under the hospital condition have been analysed with respect to free and protein amine acids by the paper chromatographic technique.

The lipid content of whole blood from both the normal and the leucoderma patient was studied. The total lipid was further fractionated into phospholipids (lecithin, cephalin), cholesterol. The significance of these data will be assessed after an adequate number is studied.

Clinical.—Majority of cases coming for leucoderma (Vitilige) show some sort of gastro-intestinal disorder and clinical investigation reveals intestinal infection by one or more of the pathogenic organisms (other than bacteria). On the basis of gastro-intestinal disorder cases have been grouped into :—

A. Cases with definite colitis having specific pathogenic intestinal infection.

B. Cases with no clinical manifestation of colitis.

Other precipitating factors associaied with the appearance of the lesion are trauma, application of some sort of cosmetics etc.

Response depends on the treatment of specific infection, type of lesion, duration and finally on the local pigment stimulating applications.

Muco-cutaneous and cosmetic types and late cases do not reveal satisfactory response.

Good response has been obtained in generalised type with shorter duration or in those appearing shortly after gastro-intestinal attack.

Although apparent, the role of specific treatment, nutrients, high protein, essential amine acids and hepatic tonics etc., will be assessed at the conclusion of the experiment.

Group.	Total number of cases.	Response.		
		Complete	Partial or satisfactory	Poor.
A	15	2	9	4
B	7	–	5	2

12. Enquiry on synthetic media of tissue culture and the measurement of proliferation of cells under Dr. C. V. Ramakrishnan at the Faculty of Science, Baroda University, Baroda.

The project on tissue culture was started in this laboratory with the following objects :—

(i) To standardise methods for the measurement of uptake of nutrients by a growing tissue speck. (ii) To standardise methods for measuring tissue proliferation. (iii) To study the enzyme systems active in the tissue and their change with growth or deterioration. (iv) To differentiate proliferating surviving and deteriorating tissues with the help of nutritional and biochemical data and to differentiate tissues which can not as yet be differentiated on a histochemical basis.

It has been possible to achieve standardization of the conditions for growing chick embryonic tissues directly on glass in roller tubes in a purely synthetic medium.

Micromethods have been standardised for the estimation during the proliferation of the tissue speck in synthetic medium, of sugar uptake, (Gothoskar, Ratnam and Ramakrishnan, Clinica Chimica Acta, 1995), increase in tissue protein (Gothoskar, Ratnam and Ramakrishnan, Naturwissenchaften, 1958), and amino acid uptake (Gathoskar, Raina and Ramakrishnan, communicated)

Micromethod for the estimation of respiration and enzyme activity in the proliferating tissues, with special reference to the Kreb cycle enzymes have also been worked out.

(Gothoskar, Raina and Ramakrishnan, paper under preparation).

It is found that the ratio of change in tissue protein to change in sugar content of the medium yields a measure that enables us to differentiate proliferating, surviving and decaying tissues from one another (Gothoskar, Ratnam and Ramakrishnan, Ind. Jour. Med. Res. 1958; Gothoskar, Ratnam and Ramakrishnan, Experimental cell Res. 1958). Preliminary investigations carried out show that chick tissue does not need glutamine, whereas rat and human tissue are known to need glutamine (Gothoskar, Ratnam and Ramakrishnan. Ind. Jour. Med. Res. 1958). This observation derives additional reinforcement from the high content of glutamine synthetase enzyme in chick tissue and low content of the same in rat tissue. It is also found that the ratio of protein synthesis to sugar uptake differs in various media. (Gothoskar, Ratnam and Ramakrishnan, Ind. Jaur. Med. Res. 1958). These findings underline the need for obtaining precise biochemical data on various normal tissues and for working out standard conditions for their growth in synthetic media.

13. Clinical study of neuropathies under Dr. P. N. Chuttani at the Medical College, Amritsar.

A total of 261 cases of various types of neuropathies were studied first at Amritsar, later at Patiala and now again at Amritsar. 251 cases have been analysed in detail, the remaining 9 did not stay long enough for a thorough study and one died on the 3rd day of admission. The main aetiological or clinical groups met with were diabetic neuropathy in 86 patients, arsenical neuropathy in 23, Landry-Guillain-Barre syndrome in 24, subacute combined degeneration in 18, meralgia paraesthetica in 21, shoulder girdle syndrome in 7, nutritional neuropathy in 14, and the "idiopathic" variety in 20. A large number of other varieties were also seen such as those due to diphtheria (4), leprosy (6), typhoid (3), serum or post-vaccinal (3), alcohol (3), compression (2), pneumonia (1), dog bite (1), quinine injection (1), brucellosis (1), bronchogenic carcinoma (1), cancer penis (1), acute idiopathic porphyria (1), hyperemesis gravidarum (1), carbon monoxide (1), amyloidosis (2), cranial polyneuritis (3), neuropathy associated with pink disease (1), polyarteritis nodosa (1) and chronic progressive neuropathy (1).

Apart from routine laboratory investigations, nerve biopsies from digital nerves of the involved limbs were available for histopathological examination conducted after staining of paraffin sections with H. & E., Van Geison, osmic acid or myelin stain, azocarmine or P.A.S. and of frozen sections with silver stain. Frozen sections were also studied under polarised light. Changes such as increased cellularity, thickening of perineurium and/or endoneurium, increase of fibrous tissue, fragmentation, granularity, ballooning and absence of myelin and swelling, fragmentation, diminution or absence of axons were seen in various combinations. No specific histopathological picture emerged for any particular aetiological variety and there was no obvious correlation between the severity of histological change and the clinical condition. However, pathological changes were the rule when neuropathy was well developed. Seventyfive cases reported for one or more follow-up examinations. Serial nerve biopsies were done in twenty patients at intervals, longer than two months. While in five instances repeat biopsies showed severer changes, improvement was noticed in three ; others showed no appreciable change.

14. Inquiry entitled 'Relative value of rest and movement in the treatment of intra-articular fractures-an experimental study' under Dr. B. Mukopadhya at the P.W. Medical College, Patna.

During the current year experiments were limited only to the knee joint. It was decided to produce damage to individual structures of the joint, as for example, the articular cartilage, the cancellous bone, the synovial membrane, etc. and then to study the effect of each of these injuries on the structure and function of the joint. With these objectives, the damage was limited to the articular cartilage in the first series of experiments, and in the second series only a linear transverse fracture of the patella was produced without damaging its tendon. Following the injury one series of animals was immobilized in plaster while the other series was not so immobilized. The function of the joint was assessed accurately every day and the results noted. After sacrificing the animals at fixed intervals from the date of operation, a complete and thorough examination of the damaged joint was carried out. Photographs were taken at the time of sacrifice and careful histological examination was made of all layers of tissues.

The following conclusions were drawn :—

(1) The articular cartilage when damaged by intrarticular fractures, undergoes a distinctive type of repair. The area denuded of articular cartilage is covered by an exudate in which there is proliferation of fibrous tissue cells. Study of the material at different intervals from the date of injury indicates that this exudate which fills up the denuded area is gradually invaded by a type of granulation tissue. The deeper parts of this granulation tissue which lie in contact with the subjacent bone undergo a process of gradual replacement by fibro-cartilagenous cells. These cells are probably derived by cell metaplasia in exposed bony trabucale. The marrow in this area undergoes considerable proliferation with the presence of a large number of newly formed blood vessels. Gradually the whole area of denuded cartilage comes filled up with fibro-cartilagenous tissue. The thickness of this fibro-cartilage is not uniform nor are the cells arranged in any regular pattern. At the margins of the denuded area of the articular cartilage the repair may not be as advanced as in the central portions. Gradually, the superficial layers of this fibro-cartilage become flattened and the surface layer comes to resemble the surface layer of normal hyaline cartilage. As time goes on further changes can be noticed. These consists of gradual transformation of the fibro-cartilage into hyaline cartilage in the superficial portion. The deeper portions undergo gradual ossification, the process of these changes being a direct transformation of fibrous tissue elements into bony tissue. Ultimately at the end of 18 weeks, which is the longest period for which these animals have been observed, the damaged area of the articular cartilage is covered by tissue which very cosely resembles normal hyaline cartilage. So far as has been observed, it has not been possible to detect any difference in the nature and type of repair between the animal which had post-operative immobilization and those which did not have such treatment.

Interesting as the changes in the denuded area of the articular cartilage are, the changes which occur in the articular cartilage of the con-

dyle of the femur, over which the damaged area of the patellary surface plays during movements of the knee joints, are even more interesting. The changes in this area may be summarised as of two types.—

(a) The more frequent changes consist of degeneration, vacuolation, hyalinization and destruction of the articular cartilage cells. The superficial layers of the articular cartilage are shed off and the cartilage becomes thinned so that in certain areas the subchondral bone may become exposed. In this degenerated portion of the cartilage there is evidence of fibrosis and also formation of fibro-cartilagenous cells. The subchondral bone also shows definite changes. The predominant picture is that of suppression of the normal articular cartilage. The cells lose their regular columner arrangement and their normal pattern is disturbed.

(b) In a smaller proportion of cases, although there is some degeneration and vacuolation of the articular cartilage cells the predominant picture is that of proliferation so that the surface layer of cells is considerably thickened. However, even in these cases there is disturbance in the smooth lining of the surface layers.

(2) In the series of experiments in which a simple linear fracture through the whole thickness of the patella was created, with or without subsequent immobilization, the following changes were noted :—

Microscopically :

(a) There was some evidence of fibrosis in the synovial membrane.

(b) There was definite evidence of a rusty pigmentation of the synovial tissues.

(c) The fracture line in the articular cartilage could be detected even at the end of 12 weeks—the longest period for which these animals were observed.

(d) No evidence of the line of fracture could be detected in the bone even at three weeks after the experiment.

Microscopically, the changes are very interesting. In all animals so far observed, the bone had healed completely so that no evidence of the fracture line could be detected in any section taken three weeks after the operation. The articular cartilage, however, showed presence of a slit like gap in every case except one. The margins of the slit were smooth and lined by cells resembling the cells of the surface layer of the articular cartilage. At the deepest part of the slit where the cartilage joins bone, there was evidence of fibrous proliferation and the bony trabeculae adjacent to this area showed definite evidence of osteoblastic activity. Only in one case the area of damage in the cartilage was filled up with fibrous tissue. The articular surface of the femur showed minimal changes. The histological appearances did not differ materially between the two groups of the cases.

In the group of animals in which pure articular-cartilage-damage was produced, the function in the limb returned in an average period of 8 to 9 days from the date of the operation if no immobilization was instituted. In the group of animals where the operation was followed by a period of three weeks immobilization in plaster, full function returned on an average about 14 to 15 days following the removal of the plaster. Thus, the total period of disability in this series amounted to an average of five weeks instead of 9 days as in the non-immobilized group. Exactly the same results were obtained in the group of animals in which a transverse fracture of patella was produced. Thus, it will be seen that where the articular damage is minimal restoration of function is complete whatever the method of treatment adopted. This is in accordance with the results of our previous experiments. The only advantage obtained by not immobilizing the limb was that complete functional restoration was achieved in a shorter period of time. Histological studies also indicated that the nature of tissue repair in groups immobilised and not immobilised are similar and no difference could be found in the local tissue changes. Further experiments with more severe types of injury to the joints are in progress.

15. Experimental study of the role of adrenal cortex in the genesis of congenital abnormalities under Dr. I. P. Agarwal at the G. R. Medical College, Gwalior.

The experimental study was carried out on two different breeds of fowls i.e., Rhode Island Red (R.I.R.) and white leg-horn (W.L.H.) in order to evaluate the effect of genetic constitution in relation to the role of adrenal cortex in the genesis of congenital abnormalities. The chick embroys of R.I.R. showed a high incidence of spontaneous early deaths and congenital abnormalities (29 per cent and 19.8 per cent respectively), while the chicken of pure breed W.L.H. fowls showed 11.5 per ce ıt spontaneous deaths and 2.2 per cent congenital abnormalities. The former probably had an inherent weaker genetic background.

The main conclusions of the present study are as follows :

A. *Action of cortisone acetate on embryonic tissues of the chick* (Injection of 0.0048, 0.0032 and 0.0016 mg. per egg into the albumin of egg)—

(i) Cortisone acetate even in such minute doses exerted a very marked lethal action on embryonic tissues of the chick. This action is independent of genetic constitution of the two breeds.

(ii) Cortisone acetate exerted teratogenic action only in R. I. R. which had a defective genetic background as evidenced by a high incidence of spontaneous congenital abnormalities. In pure breed W. L. H. which showed few congenital abnormalities, cortisone failed to manifest any teratogenic action. *Therefore, it will be more logical to state that cortisone potentiates the expression of inherent genetic weakness.* This is in eonfirmity with the observations of Fraser, Fainstat and Kalter who observed increased incidence of cleft palate after cortisone in a strain of mice genetically susceptible to this condition. This can also explain the failure of many workers to demonstrate any teratogenic action of cortisone acetate in chicks, rats and mice while others observed the potentiating action of cortisone acetate on spontaneous abnormalities in mice, insulin induced bnormalities in chick embryos and hyper-vitaminosis A indu. a d abnormalities in rats.

B. *Further investigations into the anti-teratogenic action of cortisone acetate.*

Injection of cortisone acetate into mother hens of W. L. H. breed, did not produce any effect on egg laying capacity, fertility, spontaneous early deaths of chick embryos but on the other hand, eliminated congenital abnormalities. Since this breed has not got a very low incidence (2.2 per cent) of spontaneous congenital abnormalities it is not possible to evaluate the exact significance of this observation. However, this assumes further importance in view of the observations made earlier that cortisone acetate has been successful in eliminating the teratogenic action of trypan blue on rat embryos.

C. *Comparative study of congenital abnormalities induced by cortisone, insulin and eserine sulphate.*

It appears that teratogenic effect af insulin is also partially dependent upon the inherent genetic weakness of individual breed. This qualitative similarity of insulin and cortisone (both being internal secretions) requires further study.

D The histological examination of adrenal gland in controls and cortisone treated chick embryos did not show any change in morphology and distribution of sudanophilic material.

E. Vitamin C has also been estimated in allantoic fluid of chick embryos in control series of both the breeds.

16. Investigations into the epidemiological factors of rheumatic heart disease under Dr. Devi Chand, and antistreptolysin titres in children under Dr. S.L. Bhatia at the Lady Hardinge Medical College, New Delhi.

Main objective of this enquiry is to make a complete study of the epidemiological problems connected with rheumatic fever and rheumatic heart disease in India. The enquiry was originally started in the Hills of Himachal Pradesh in 1957. During the first year the incidence and epidemiological factors operative in the Himalayan Hills were studied and reported. During the current year the enquiry was continued at Delhi, and besides studying the epidemiological factors in the plains, attention was foccussed on clinical mainfestations, the role of heridity, the incidence of streptococcal and nonstreptococcal trigger machanisms and the serial antistreptolysin O titres in patients suffering from rheumatic disease as compared to suspected rheumatic and nonrheumatic patients. A study of streptococci from throat cultures was carried out is being specially runstudies were also undertaken. A rheumatic clinic and C-reactive protein for this project at the Irwin Hospital New Delhi.

Bacteriological work connected with the enquiry was carried out by Dr. S. L. Bhatia, Professor of Bacteriology, Lady Hardinge Medical College, New Delhi.

So far 162 patients of rheumatic fever and rheumatic heart disease were studied in Delhi. Of these 130 were proved cases of rheumatic disease and 32 were doubtful cases. The antistreptolysin O titres were done in all of them. However, detailed clinical analysis could be made only in 115 of the 130 proved cases. The results are given below:—

Clinical.—There were 70 males and 45 females. Two were under 5 years of age, sixty-eight between 6 and 20 years, forty-four between 20 and 40 and six above 40 years of age. Eleven cases presented themselves in the classical phase, 99 in the phase of protracted carditis and 5 in the quiescent phase. Among cases of protracted carditis 62 had active rheumatic process at the time examination, and of these 54 were in congestive heart failure. History of classical attack could be elicited in only 53 per cent of the cases while in the rest the onset was either with low grade symptoms or the lesions were discovered accidentally on routine physical examination. Whatever the mode of onset, more than 70 per cent of the cases had their first symptoms between 6 and 20 years. An antecedent history revealing the association of trigger machanisms prior to the onset of rheumatic syndrome could be elicited in 46 cases. This was a more common feature in cases who had the onset with classicsl rheumatic attacks. However, out of 30 cases of this type a history of sore-throat and upper respitatory infection could be obtained only in 8 cases. The other precipitating factors elicited were : bacterial infections—2 cases; fever of undefined nature—5 cases; exposure, fatigue, exhaustion and chill-6 cases; physical trauma—3 cases; pregnancy or delivery—2 cases and acute psychic stress—3 cases. This emphasises the role that non-streptococcal trigger machanisms play in exciting the rheumatic syndrome.

Regarding clinical mainfestations, the majority of cases had arthralgias and 11 had actual arthritis. Eight cases had chorea. No case with subcutaneous nodules or erythema marginatum was seen. All cases showed evidence of carditis. Significant apical murmurs were present in 111 cases. The incidence of valvular involvement was as follows:-

Mitral senosis, 18; mitral incompetence, 30; mitral stenosis and and incompetence, 48; aortic stenosis, 5; aortic stenosis and incomptence, 2; pure aortic incompetence, 1 and combinations of mitral and aortic lesions, 10 cases. One case had tricuspid regurgitation.

Bacteriological.—The normal antistreptobysin titre was 200 units as indicated by results on a majority of controls. All cases in the classical phase had a titre above 400 units, most of them showing a titre above 1000. Amongst cases with protracted carditis showing signs of activity, eleven showed a titre of less than 200, another eleven between 200-400, four between 400-1000 while in one case it was above 1000. In cases of protracted carditis without rheumatic activity seven showed a titre of below 200, three between 200-400, 9 between 400-1000 and two above 1000. In the absence of rheumatic activity in this group, the high titres observed seem to be contrary to expectations. Among the controls, the majority showed titres below 200 units, fourteen between 200-400, two between 400-1000 and in two cases the titre was above 1000. Investigations which are in progress on the lines indicated above will throw further light on the epidemiological and clinical aspects of the problems.

17. Comparative study of serological techniques in detecting antibodies following immunization with S. Typhi O antigen in rabbits and in sera of patients suffering from enteric fevers under Dr. A.K. Banerjee at the S.S.K. Memorial Hospital, Calcutta.

S. typhi (0.901) collindale strain has been used in this investigation. The O antigen of the intact bacteria and the isolated soluble polysaccharide fraction of the O antigen complex, have been studied. The studies on the polysaccharide fraction also included quantitative chemical analysis and estimation of its sugar. The serological studies included qualitative bacterial agglutination, polysaccharide haemagglutination, immune-haemolysis and their specific inhibition by the polysaccharide heptene.

The organism has been adapted to grow in a synthetic medium. Work is in progress to study the serological behaviour of antibodies following immunization with antigen (S. typhi-O) containing known amounts of bacterial nitrogen, lipid, polysaccharide and an appropriate mixture of them. Serological techniques in saline and macro-molecular media are being used in the study of both rabbit and human sera.

18. Enquiry on the early diagnosis of enteric fevers under Dr. N.P. Gupta at the K. G. Medical College, Lucknow.

Laboratory diagnosis of enteric fever requires 48–72 hours with the available procedures like blood culture, Widal test, stool culture, etc. It is even more difficult in early stages of disease when only blood culture is said to be positive. With the rapid advance in the treatment of enteric fever, it has become necessary to discover techniques to diagnose these cases early and quickly. It was proposed to investigate if a soluble antigen could be detected in blood and/or urine in early stages of this disease in human beings and in experimental mouse typhoid.

(i) Attempts were made to detect the presence of soluble antigens or haptens in blood and urine of patients by haemagglutination inhibition bacteriophage inhibition and complement fixation tests.

Eight patients with a positive blood culture on admission, were studied and their urine was examined for a number of days for soluble antigens. Haemagglutination inhibition was observed in the urine of 5 out of 8 cases of enteric fever investigated. This inhibition was observed in the second week of the disease in all the 5 cases and sometimes even after the agglutinins appeared in the blood.

Haemagglutination inhibition test was positive with the serum of two out of 16 cases of enteric fever with positive blood culture. In these cases complete inhibition of haemagglutination was observed.

The bacteriophage inhibition test was carried out by noting a reduction in the number of plaques obtained with a standard V phage A, which had been incubated with urine or serum. Parallel titration of original phage was also carried out. This test was done with urine of 8 cases and serum of 7 cases of enterio fever. No reduction was seen in the number of plaques. It was concluded that the Vi antigens or haptens were probably not present in the urine or serum in a soluble form.

Complement fixation test by using serum of patients as antigen and standard antiserum of Salmonella typhi 'O' as antibody, was done with serum of 8 cases of enteric fever at the time when bacteraemia was present, but it was not possible to demonstrate the presence of soluble antigen by this method.

(ii) Observations were also made on experimentally produced mouse typhoid with S. typhi-murium. Four sets of experiments were done. Two sets of 50 mice each were infected by intraperitoneal injections of S. typhi murium, another 2 sets of 100 mice each were infected by oral feeding with the same organism. Mice were kept in metabolic cages, and haemagglutination inhibition test was done with the urine and faecal extract of mice in all the 4 sets throughout the course of the disease. During the course of disease in all the experiments, phases of bacteraemia were recorded by repeated bacterial counts in the blood of infected mice.

The haemagglutination inhibition test was significantly positive with urine of infected mice after the appearance of secondary bacteraemia in all the four experiments. Haemagglutination inhibition test with faecal extract in all the four experiments did not give any positive results. Haemagglutination inhibition test with serum of infected mice also did not show any positive results.

19. Investigation into the problem of chronic splenomegaly and its relation to hepaticpathology under Dr. B. K. Aikat and Dr. A. K. Basu at the S.S.K.M. Hospital, Calcutta.

The object of this enquiry was to study the (a) the association of chronic splenomegaly with hepatic pathology and (b) the effect of splenomegaly on the structural and functional status of the liver.

The study included :

A. 1. Investigation of cases where splenomegaly was one of the outstanding features to establish the cause or causes of such splenomegaly. Investigations into the functional and structural status of the liver in such cases.

2. A study of selected cases on whom splenectomy was performed with or without an appropriate shunt operation-

3. A longitudinal study of such cases involving assesment of the clinical, haematological and the structural and functional status of the liver was undertaken during the post-operative follow-up period.

B. Experimental production of hepatic damage with or without gross splenomegaly and study the effect of (i) splenomegaly on the evolution of the hepatic lesion and (ii) splenectomy on the evolution of experimental cirrhosis, performed at various stages.

Results of the Clinico-pathological study :

Analysis of 75 cases of Splenomegly.

Aetiolo ical classification.		*No. of cases.*
1. Primary Liver Disease :		
(a) Post necrotic cirrhosis	..	26
(b) Diffus Hepatic Fibrosis	..	12
(c) Postnecrotic scarring	..	3
(d) Late stages of cirrhosis anatomically unclassified	..	11
		52

2. Extrahepatic Obstruction	..	13
3. Thalassaemia E. Disease	..	3
4. Chronic Malaria	..	3
5. Kala-azar	..	2
6. Polycythaemia Vera	..	1
7. Primary Splenic Tumour	..	1
		75

After assessing the functional status splenectomy, with or without splenorenal shunt was performed in the following groups :—

	Splenectomy with shunt	Splenectomy alone
Post necrotic cirrhosis ..	7	11
Diff. Hepatic Fibrosis ..	4	5
Post necrotic scarring ..	3	0
Ext. Hep. Obstruction ..	4	8
Primary R.E. Hyperplasia	0	4
Total	18	28

The subsequent assessment has been done as a longitudinal study during the follow-up period of 3 years.

Conclusions from the Clinico-pathological data.

1. In every case of splenomegaly the cause could be established. It appears that there is very little justification in recognising any further, the term, "Tropical Splenomegaly".

2. Hepatic pathology constituted the commonest aetiological factor. Post-necrotic cirrhosis and scarring formed the largest single group.

3. No linear relationship was detected between the extent of portal pressure, ascites, haemmorhagic episodes and the size of the spleen.

4. The largest spleens were seen in the post-necrotic cirrhosis and scarring group as also in primary R.E. cell hyperplasia of the spleen. Histologically, cellular hyperplasia was the most common feature in splenomegaly associated with liver disease. The size of the spleen was comparatively smaller in the extra-hepatic obstruction group where the classical picture of fibrocongestive splenomegaly was seen.

5. Haemmorhagic episodes were the most frequent symptoms in the extra-hepatic obstruction group in whom absence of ascites, however, was the outstanding feature.

6. A hypersplenic factor was observed in all cases and in almost every case there was improvement of the haematological status after removal of the spleen.

7. Although the number of operated cases with reasonable duration of follow-up study is limited, it appears that in properly selected

cases splenectomy with shunt has given the most encouraging results in all the studied groups. Splenectomy alone appears to be less effective and carried a higher mortality rate during the subsequent follow-up period. In the primary liver diseases group, the results were most satisfactory in post-necrotic scarring. Comparatively, the post-necrotic cirrhosis group have shown better posts-operative results than cases with diffuse hepatic fibrosis.

8. One of the important complications during the post-operative period was infectious hepatitis confined only to the post-necrotic cirrhosis and scarring group. The cause of this selective incidence may be of significance although can only be a matter for speculation at this stage.

9. Pathology of the spleen:—Contrary to the existing concepts, a marked difference was observed between the pathology of the spleen complicating liver disease and those due purely to mechanical obstruction due to extra-hepatic obstruction. While the classical picture of fibrocongestive splenomegaly was observed in the later group, reticuloendothelial hyperplasia was the most prominent feature in cases with cirrhosis. Such cellular hyperplasia could be well compared with primary R.E. cell hyperplasia associated with conditions, like Kala-azar, Malaria or Thalassaemia E. disease. The congestive features were very much less marked both in extent and in distribution. Similar congestive features were also seen in Primary R.E. hyperplasia of the spleen.

Experimental Study :

A pilot study was first carried out to produce signficant degree of splenomegaly by intra-peritoneal injections of a macromolecular substance, methylcellulose with a viscosity grade of 400 centipoises. It was possible to produce gross splenomegaly by giving 30 injections of 1 c.c. of 2.5 per cent methyl cellulose intra-peritoneally twice a week.

Liver injury is being produced by 30 biweekly injections of carbon tetrachloride of 0.1 c.c. per 100 g. of body weight in adult albino rats.

Experimental groups under investigation :

Group A	Ccl_4 with Bal. diet	Ccl_4 with 2 per cect prot. diet.	Ccl_4+Meth. Cell.+ Bal. Diet.	Meth. Cell+ Bal. Diet.
Group B	Splenectomy in each of the above groups after 30 injections.			
Group C	Primary splenectomy followed by the procedures in Group A.			

The structural and functional status of the liver is being evaluated in each group along with biochemical estimations of the liver tissue.

20. Inquiry into electrophoretic study of immunised rebbits with particular reference to immunological tolerance under Dr. D. Barua at S.S.K.M. Hospital, Calcutta.

The object of this part of the study was to see whether acquired immunological tolerance could be established in rabbits with bacterial antigens and to see whether such tolerance could be correlated with the synthesis of gamma globulin by baby-rabbits.

Rabbits of unselected breed were mated in the laboratory and their offsprings were used for the experiments. A heat:killed suspension of localy isolated strain of *S. typhi* was used throughout the experiments for immunisation. For agglutination reaction the 'H' & 'O' suspensions were prepared from standard strains of *S. typhi* obtained from NTCC, Colindale. The sensitivity of each of the suspensions were tested with standard were before being used for the tests.

Six adults rabbits which were free from antibodies against *S. typhi* were immunised by one 1M injection of *S. typhi* antigen. Four weeks after the injuction the average agglutination titre against TH was found to be about 1:320 and against 1:800.

Only 10 baby-rabbits born of 4 mothers free from antibodies against *S. typhi* survived the experiments which is associated with high mortality rate. One out of each of 4 litters was kept as control and remaining 6 were immunised with 0.2 c.c. of the antigen containing about 50x106 organism injected intramuscularly within 4-16 hours of birth. Two and four weeks after immunisation, the sera of the control and test animals were found to contain no demonstrable antibody.

These ten animals were challanged again with the same antigen when they are about 16 weeks old as in case of the adult control animals.

Four weeks later the titre of the sera of the control baby rabbits was found to vary between 1:160 and 1:1280 and that of the test animals from 0 and 1:160.

Eight pregnant rabbits, free from antibody against *S. typhi* were opened up on the 25th and 30th days of gestation and the embroys in utero were injected lm with 0.2 c.c. of the antigen containing about 50 x 106 organisms. Only 3 baby -rabbits of 3 litters which were immunised on the 30th day of the intrauterine life survived. Two and four weeks after birth, these animals showed no antibody in their sera. They were immunised again at their 16th week of life by one lm injection of the same antigen containing about 260x106 organism. Four weeks later the titre was found to vary between 0 and 1:80.

These experiments tend to show that partial immunological tolerance could be established in rebbits with *S. typhi* antigen.

Electrophoretic study of the sera of new-born rabbits revealed presence of gamma-globulin in their sera in varing amounts which may be borrowed gamma- globulin from their mothers. So investigation in this line was given up.

Attempts were then made to see wheather immunological tolerance could be established with bacterial antigen in monkeys as the period of gestation of monkeys is almost of the same duration as in man.

Four pregnent monkeys were available to us. Within a few hours of their birth, 3 baby monkeys were immunised with a single 1 m injcetion of *S. typhi* antigen (about 100 x 106 organism). One of the monkeys died.

Two weeks later their sera were found to agglutinate the TH & TO suspensions and their titre varied from 1:80 to 1:320. The sera collected at the time of birth from the test animals and on the 18th day from the uninoculated baby monkey did not have any antibody. This shows that monkeys are immunologically more mature at the time of their birth and immunological tolerance cannot be produced by exposing them to antigens of this nature at that time.

21. Study of Kwashiorkor with special reference to histo-pathological and histochemical changes in the cutaneous lesions under Dr. C. Mohan Rangam at the M.G.M. Medical College, Indore.

The material studied so far consists of skin biopsies from 21 children with typical cutaneous lesions of kwashiorkor and 10 normal healthy skins.

The criteria for selection of cases and the histo-pathologica l patterns observed in the skin biopsies, studied by various staining techniques have been reported earlier. Further studies conducted along the same lines this year confirmed our earlier findings, which are.

(1) the distribution of the cutaneous lesions in kwashiorkor does not differ from that in pellagra,

(2) the epithelial atrophy may co-exist with hyperkeratosis, and parakeratosis in lesions of same age,

(3) it is possible to correlate the clinical appearance of any particular type of lesion with specific histopathological changes, e.g.,
   (a) parchment dermatosis with uniform and marked epithelial atrophy.
   (b) crazy-pavement dermatosis or enamel paint dermatosis with co-existent hperkeratosis, parakeratosis and atrophy.
   (c) crackled skin appearance with marked corrugation of the epidermis.

In addition, in 4 of the cases in this series there was a striking degree of keratinization of hair follicles and in the majority of cases an extreme paucity of sweat and sebaceous glands was observed. However, when the latter did exist, an appreciable amount of histiocytic infiltration was found around them.

The skin biopsies were also studied histochemically for their content of alkaline phosphatase by Gomori's method and for their content of total and differential nucleoproteins by Toluidin Blue and Pyronin Methyl Green techniques, respectively. Vitamins A and C have been estimated quantitatively in a few of the biopsies.

In all but 4 cases there was a striking diminution in the total nucleoprotein content. Both D.N.A. and R.N.A.showed appreciable diminution but the diminution in the latter was much more pronounced. All the cases showed a striking increase in the alkaline phosphatase content in the epidermis. Unlike the controls where it is seen as fine granules diffusely distributed in all the layers of the epidermis, in kwashiorkor it seems to be concentrated maximally in the stratum germinativam producing a band-like appearance.

Attempts to demonstrate vitamin A histochemically by the Carr-Price reaction and to estimate it in the homogenised tissue by the method of Pet t and Lepage have not been successful. Similarly, histochemical demonstration of vitamin C by Bourne's technique was not possible but quantitative estimation of this substance in the skin showed significant reduction as compared to normal skins. In these cases, reduction in blood vitamin C levels was also observed.

22. Studies on the role of inositol in hyperlipemic conditions under Dr. V. Srinivasan at Madurai Medical College, Madurai.

Preliminary studies showed that Cholesterol, particularly the free form, accumulated in the tissues of the organism Coryra Cephalonica st. and also in the blood of the albino-rats fed on inositol deficient diets. In these studies gammexane, an inositol antimetabolite was used for producing the inositol deficiency. Based on the results of these studies experiments were conducted to elucidate the biochemical function of this vitamin in cholesterol metabolism with special reference to its influence on the cholesterol esterase activity of liver and pancreas. The preliminary *in vitro* studies led to the interesting finding that inositol almost completely reversed the inhibitory effect of gammexane on this esterase. This *in vitro* study was next extended to *in vivo* studies in albino-rats, where the liver cholesterol esterification was studied in rats maintained on different types of rations which are known to produce fatty livers, such as high cholesterol feeding, high fat feeding, pyridoxine deficiency, pantothenic acid deficiency and choline deficiency. Free cholesterol was markedly increased under most of the experimental conditions used. Cholesterol esterase activity was found to be low in all inositol deficient groups and curative studies with inositol brought the enzymic level in the liver to normal. During these studies it was frequently noticed that the adrenals showed marked enlargement in inositol deficiency. Therefore, the role of inositol in adrenal cortical function is also being studied to see whether this vitamin has any enzymic role in the biosynthesis of the adrenal steroids.

23. Study of the effects of selected respiratory stimulants in states of pulmonary insufficiency associated with hypercapnia and hypoxia under Dr. N.R. Konar at the Nilratan Sircar Medical College, Calcutta.

Thirtyone patients with pulmonary insufficiency, diagnosed clinically were selected for studies. In addition to a detailed clinical survey, respiratory function tests, including spirometry and arterial blood gas analy- ses were done before and after administration of a respiratory stimulant. In 23 patients, the tests were carried out before and after intravenous administration of o.24 Gm. of Aminophylline and in 8 patients before and after inhalation of oxygen for 20 mins. Besides these, Oximeter was calibrated against Vanslykes manometric apparatus and maximum breathing capacity test was carried out in 51 normal subjects.

The maximum breathing capacity (M.B C.) in normal subjects was observed to be 116 lit/min B.T.P.S. (mean value). Statistical analysis showed that the value of M B.C. varies inversely with age and directly to surface area of a subject. From this study, it has been found out that the M.B.C. can be predicted in 90 per cent of cases with+20 per cent accuracy from the following formula :—

Y=106.85+(30·93 × surface area in sq m.)—(1·21 × Age in yrs.)
Where Y=predicted M.B.C. of a male subject in standing posture.

It was observed that emphysema of lungs, either due to chronic bronchitis or bronchial asthma was the main disease which caused pulmonary insufficiency. In some of the patients, emphysema produced congestive cardiac failure, The rest were patients suffering from bronchopneumonia and tropical eosinophilia. (Patients suffering from pulmonary tuberculosis were not included in this study due to practical difficulties). Majority of the patients were between 35 and 45 years, the mean age being 41 years. Most of the patients investigated in this series had chronic diseases of lungs, mean duration of illness being 6 years. The symptoms that were present in patients with pulmonary insufficiency were, dyspnoea of various degrees, cough, fever, pain in the chest, palpitation and oedema in dependant parts. 90 per cent of patients investigated gave a past history of some lung disease.

Regarding respiratory function tests, it was observed that pulmonary ventilation was high in patients with mild pulmonary insufficiency and it was low in patients with severe pulmonary insufficiency. Maximum breathing capacity, as well as the vital capacity, were grossly diminished in patients suffering from obstructive lesions in pulmonary tree. Dyspnoeic index was below 65 per cent in 55 per cent of cases. Air velocity index was less than 1 in obstructive lesions of lung. Timed vital capacity was diminished with prolongation of the time for expiration, in a majority of the cases investigated.

Arterial blood showed anoxaemia and hypercapnoea of different degrees.

Slow intravenous injection of o.24 Gm. of Aminophylline resulted in an increase in pulmonary ventilation. This was associated with bron-

cho-dilatation as evidenced by increase in M.B.C. and vital capacity. There was a slight increase in oxygen content and slight diminution of carbon-dioxide content of arterial blood in most of the cases. This change in oxygen and carbondioxide content in arterial blood was, however, not statistically significant. Oxygen consumption determined in 10 cases after the injection of aminophylline showed an increase in 4 cases and no change in the rest.

Inhalation of oxygen for 20 mins. showed no effect on ventilatory function tests but anoxaemia was corrected to a great extent in all the cases. Arterial blood analyses showed an increase in oxygen content of the arterial blood in all the cases, but in 62 per cent of cases the arterial oxygen saturation did not come up to the normal level. In cases of severe anoxaemia the rate of oxygen consumption was observed to be very rapid at first and was rather steady in later stages when anoxaemia was corrected to some extent. After oxygen inhalation, the carbondioxide content of arterial blood, was raised in only one case out of eight and this was not attended with any diminution of pulmonary ventilation.

24. Inquiry on the pollination calender for Greater Delhi at the Vallabhbhai Patel Chest Institute, Delhi

Botanical field survey was carried out throughout the year to complete our list of allergenic plants of Delhi and to note their pollination seasons. In addition to the list already submitted three more plants namely *Ricinus communis*, *Putranjiva roxburghi* and *Dodonaea viscosa* were found to produce and shed abundant amount of pollen. Over 100 plants were collected to serve as herbarium and about 175 reference slides were made to facilitate identification of atmospheric pollens.

Atmospheric pollens were studied and recorded daily over a year from the roofs of (1) Patel Chest Institute (2) Lady Hardinge Medical College and (3) Victoria Zenana Hospital (near Jama Masjid). The common types of atmospheric pollens so far indentified are the following :—

(1) Grasses (2) Cyperaceae (3) Chenopodium-Amarantus type (4) Cannabis sativa (5) Prosopis (6) Pinus (7) Xanthium (8) Acacia (9) Ricinus (10) Dodonaea(11) Ailanthus (12)Acanthaceae (13) Kigelia (14) Putranjiva (15) Terminalia (16) Salvadora (17) Argemone (18) Moraceae (19) Salmalia (20) Azadirachta (21) Cruciferae (22) Compositae (23) Leguminosae (24) Myrtaceae.

During September and October grass pollens as well as those of the families of Amarantaceae and Chenopodiaceae were predominating. In November and December *Ricinus* and *Xanthium* pollens were abundant while from February to April tree pollens attain their maximum concentration. July recorded the minimum pollens on the slides.

Different methods were tried for different species to collect pollens in large amounts. About 29 types of pollens were collected and their antigens were extracted. These antigens are regularly being used for skin testing on the patients attending our asthma clinic. Those patients who give positive skin reactions are also being hyposensitized against the offending allergens. An interesting finding that has emerged out of these studies is that the pollens of plants like *Putranjiva roxburghi*, *Dodonaea viscosa* and *Carica papaya* which have not so far found a place in allergy literature could be allergenic to man.

25. Enquiry on the relative values of colpomicroscopy and vaginal cytology in detection of early carcinoma of cervix uteri under Dr. Chinmoy Ghosh at the Calcutta National Medical Institute, Calcutta.

From October 1957 to September 1958.

The programme of the work continued on the same lines as has been detailed in the previous report of the work ending September, 1957 During the year 442 patients were examined.

They are grouped as follows :—

A. only cytological examination—57 cases.

B. Cytological and Colpomicroscopical Examination—345 cases.

C. Cytological, Colpomicroscopical and biopsy examination—33 cases.

D. Cytological and biopsy examination—7 cases.

The cases according to the previous plan are further regrouped as follows :—

1. Cervix clinically healthy—231 cases.

(a) Colpomicroscopy (C.M.) & Cytology. } Negative in 172 cases.

(b) Only cytology done-negative in—57 cases.

C.M. examination could not be done in this series either on account of failure of patients to report back or due to some technical difficulties explained previously.

(c) C.M. Negative; Cytology-Positive—2 cases.

In one case smear showed endometrial type of malignant cells in a postmenopausal woman with healthy cervix which proved to be adeno-carcinoma of the endometrium on endometrial biopsy and subsequent operation.

The other case proved to be a papilliferous cyst-adenocarcinoma of the right ovary without involvement of the endometrium and the tubes.

2. Cervix clinically unhealthy—erosion, excoriation, cervicitis etc., but did not appear clinically maligant—204 cases.

(a) C.M. and Cytology Both negative—188 cases.

(b) Cytology—atypical and suspicious.
C.M. negative
Biopsy negative.
} In 11 cases.

All these patients showed active trichomonas infection.

(c) Cytology — Negative; C.M. — Suspicious } 2 cases.

In one case biopsy of the cervix revealed basal cell hyper-activity. Total hysterectomy for associated pathology and further study of the cervix revealed the composite picture of basal cell hyper activity and preinvasive carcinoma in different area.

The other patient had a normal delivery four months ago and there was no trichomonas infection. Biopsy of the servix showed basal cell activities in some areas. The case is under follow up.

(d) C.M. and Cytology— Both positive—2 cases.

Biopsy in one case proved early invasive carcinoma. The other patient did not turn up for biopsy and left touch with the unit*.

(e) Cytology, C.M. } Both suspicious—1 case.

The patient did not turn up for biopsy*

(*) Attempts are being made to contact both the cases.

3. Advanced clinical carcinoma of the cervix, including one with pregnancy—7 cases

Cytology was positive in—6 cases.

C.M. could not be done due to haemorrhage.

Biopsy-Invasive carcinoma in all cases.

26. Induction of gall stone in monkeys under Dr. B.N. Balkrishna Rao at the Medical College, Gwalior.

F.B. nuclei (zinc, magnesium and pieces from human gall stones) were implanted in the gall bladder of monkeys for varying periods, the longest post-implantation period being 986 days. The human gall stone nuclei and magnesium nuclei were completely dissolved in the gall bladder. The zinc nuclei consistently lost their original weight which shows the strong dissolving property of monkey bile. This nuclei dissolving property of the monkeys' gall bladder bile is due to its acid pH.

The failure of F.B. (zinc, magnesium and human gall stone) to incite orint stone formation points to the importance of other factors as the causative agents. It points out that stasis may be, and perhaps is, an important governing factor in the production of gall stones.

At present the effect stasis of bile—by partial obstruction of common bile duct and/or of systic duct by a silver wire—is being studied.

27. Inquiry into the indigenous materials and methods employed by the people of Andhra Pradesh for the maintenance of health, treatment and prevention of some common diseases under Dr. D. V. Subba Reddy History Osmania Medical College, Hyderabad-Dn. Research Assistant—Dr. V. V. S. Sastry.

I. *Study of printed old books in Telugu and English* dealing with herbs and drugs ; notes were taken of herbs and drugs of common use in Andhra Pradesh.

LIST OF BOOKS

1. Watt's Commercial Products of India.

2. Chemistry and Physiology of Vegetable Drugs by N.L. Allport.

3. Herbal Remedies & Receipes and some others by Quelch.

4. Waring's Remarks on the Use of Some Bazar Medicines and Common Medicinal Plants of India.

5. Pharmacopoeia of India by Warning.

6. The Useful Plants of India by Drury.

7. Dhanwantari Nighantu. (Telugu)

8. Vastuguna Prakasa. (Telugu)

9. Siddhamoolika Rahasyam. (Telugu)

10. Vastuguna Deepika, (Telugu)

II. Information gathered from *visits and enquiries among local* people :—

III. Summary of data collected.

1. *Achyranthes aspera* : Linn.—The paste of the leaves applied externally to stop the bleeding from wounds. The paste of the seeds with rice water internally for bleeding Piles. The decoction of the plant with Sal Ammonic for hepatic dropsy.

2. *Adhatoda vasica* : NEES.—For cough along with ginger, used in chronic bronchitis and asthma. Flowers and fruits are bitter, aromatic and antispasmodic. The powdered root is expectorent and antispasmodic ; used also in malarial fever, cold, cough, phtisis, diphtheria etc.

3. *Amarantus Gangeticus* : Linn.—Used as an emollient poultice.

4. *Amarantus paniculatus* : Linn.—Used for purifying the blood. Used in piles and scropula.

5. *Amarantus spinosus* : Linn.—Root is useful in menorrhagia. Plant is used as an antidote for snake poison. Root is also considered a lactogogue.

6. *Aristolochia Indica* : Linn.—Paste of the root used externally for ulcers and eczema and in snake bites and in poisonous issect bites. Decoction of the plant is diuretic and aperient, and is also useful in allergy. Root used in dysentry, fevers and leucoderma.

7. *Aristolochia Tagala* :—Used in leucoderma and in bowel complaints.

8. *Asparagus gonocladus* : Baker.—Decoction of the tubers with Tribulus Terristris in haematuria. Tubers are lactogoguic and aphrodisiac, andare given in gonorrhoea, kidney diseases, rheumatism, and nervous disorders. Juice with cow milk used in epilepsy.

9. *Asteracantha Longifolia* : NEES.—Leaves of this plant and Tribulus Terristris with cow's urine used in stone in the bladder. For sleeplessness a decoction of the root of this plant with root of Solanum Nigrum and that of Achyranthus Aspara.

10. *Azadirachta Indica* : HDR.—Juice of the leaves is used in snake bite. The paste of the leaves prepared out of cow's urine is used for skin diseases, lice, skin lesions of small-pox and chicken-pox. Leaves applied over the breasts reduce milk secretion. Flowers are anthelmintic and are also used in leprosy. Fruits are laxative, anthelmintic and used in eye diseases, and urinary diseases. Oil is used in leprosy, asthma, epilepsy and common colds. When taken in, the oil makes the hair darker. Juice of the bark with honey used in vomitings with honey and ginger in jaundice. Gum is used in dysentry and diorrhoea. Toddy that exudes from old trees acts as tonic and is supposed to be anii-bilious. Kernel of the seeds is fried with oil, ground and mixed with honey and given in severe thirst for woman in puerperium.

11. *Barleria Prionitis* : Linn.—Juice of the leaves used in fevers with lung affections. Ashes of the plant, in cough and urinary diseases.

12. *Calotropis Giganea* : R.B.R.—Tender shoots with betel leaves are given in malaria. Paste of the root with vineger applied externally in elephantiasis. Flowers used in cough and asthma. Juice of the leaves is put in the nostrils in snake bite. For pigmentation of the face, the latex mixed with turmeric is applied.

13. *Carica Papaya* : Linn.—The tender leaves are used with black papper and garlic in jaundice. Unripe fruit is lactogogue and is also used in pheumatoid arthritis. The milky juice is rubbed on the spot for scorpion sting.

14. *Centella Asiatica* : Urban.—Paste of the leaves used in leprosy. Juice of the leaves is used with milk in small-pox and chicken-pox. This plant has often been mistaken for hydrocotyl Asiatica Linn. Powder and juice of the leaves are used for menstrual diseases and syphillis.

15. *Clitoria Ternatea* : Linn.—The paste of the root with sugar, honey and ghee is used for seven days in peptic ulcer.

16. *Curculigo Orchidides* : Goertn.—Rhizome is used in spermatorrhoea, sexual weakness, deafness and the same with milk in asthma, leucorrhoea and arthritis.

17. *Datura Fastuosa* : Linn.—The seeds with long papper made into paste with cold water and given in eilephantiasis.

18. *Daucus Carota* : Linn.—Root is said to be aphrodisiac, laxative and vermifuge, and is also useful in diabetis, tuberculosis, anaemia, constipation. Juice of the root with breast milk is put in nostrils in hiccup. Leaves useful in migraine.

19. *Eclipta Alba* : HASSK.—Decoction of the leaves internally in uterine haemorrhages.

20. *Euphorbia Pilulifera* : Linn.—Juice of the leaves acts as haemostatic.

21. *Ficus Glomerata* : Roxb.—Bark is lactogogue and it also reduces sugar in urine. Fruits useful in Diabetis. Paste of the bark with breast milk is used in excessive hunger.

22. *Synnema Sylvestra* : BENTH.—Leaves are stated to be good for diabetis.

23. *Holarhena Antidysenterica* : Wall—Bark is used in bleeding Piles.

24. *Hygrophila Spinosat* : ANDERS.—Leaves are useful in jaundice and dropsy. Root is used in rheumatism. The seeds are aphrodisiac.

25. *Justicia Gendarussa* : Linn. f.—Decoction of the leaves is diaphoretic and given internally in cephalalgia.

26. *Lgcopersicon Esculentum* : Miller.—Juice of the unripe fruit is used with sugar in severe thirst.

27. *Momordica Charanatia* : Linn.—Leaves with turmeric, salt and castor-oil taken to prevent small-pox. Fruit considered useful in diabetis.

28. *Ophiorrhiza Mungos* : Linn.—The powder of the leaves is useful in scorpion sting, snake bite, hhdrophobia.

29. *Passi flora* : Linn.—The flowers are hypnotic.

IV. Information collected during the *visit to Srisailam* on the occasion of the search for herbs and exhibition of the collection by the Ayurvedic physicians.

1. *Aegle Marmelos* : barr.—The tender leaves are diuretic and used in the disease of kidney to reduce swelling of the body. The same tender leaves are supposed to supress hunger.

2. *Cressa Cretica* : Linn.—The juice of the leaves of this plant is supposed to combine with mercury and make it easily amenable to processes used in alchemy.

3. *Coleus Aromaticus* : BENTH.—It is a vermifuge.

4. *Croton Oblongifolius* : Rox B.—It is used in 'Vata' diseases and is considered as a talisman to keep the demons and evil spirits away.

5. *Gloriosa Superba* : Linn.—Root is anthelmintic and used in leprosy, snake bite and scorpion sting.

6. *Mimusops Hexandra* : Rox B.—The fruits are prepared into a drink which is a stimulant and tonic.

7. *Randia Dumetorum* : Lamk.—Fruit is emetic.

8. *Sida Acuta* : BURM.—Used in nervous diseases like paralysis and it is also a stomachic.

28. Effect of tissue therapy in the prevention of blindness due to degenerations and abiotrophies of the retina and choroid under Dr. J. Bose at the R.G. Kar Medical College, Calcutta.

When Filatov described his tissue therapy, his main target was "Retinitis Pigmentosa" or "Primary Pigmentary Degeneration of the Retina". As this disease is rather rare, help from other colleagues has been sought in the collection of cases. The number of new cases rose to twenty six as compared to only nine in the previous year. But strangely enough only one secondary case was received this year, compared to eleven in the previous year.

Before tissue therapy was started, very careful clinical examination of the eye (including records of visual acuity by special Landolt's ring chart, condition of refraction, media and fundi), laboratory investigations (e.g., W.R., Kahn, Mantoux Test, V.D.R.L.) and special examination like perimetry, dark adaptation, scotometry were completed.

Fundi were very carefully drawn by an artist with an approximate magnification of 2½ times. All these data were carefully recorded in a proforma and reserved for future use and comparison.

However, in a small percentage of cases. despite all the clinical data, definite diagnosis could not be made. Evidence of hereditary transmission is always a great help in such border line and atypical cases. As a matter of fact such evidence was obtained in 8 out of 35 Retinitis Pigmentosa cases (female–sex linked, dominant and recessive).

Tissue therapy is a long continued treatment according to Filatov's procedure but Prof. Amsler has modified this to a very short term one. A few of the difficulties in carrying out tissue therapy in our unit were irregular attendance and discontinuation of visits on the part of the patients (14 out of of a total of 35 primary cases and 5 out of a total of 12 secondary cases have discontinued their visits.)

Some young patients were deliberately not given the treatment because of repeated injections and consequent ugly scar formation. In one of them anticoagulant therapy was started.

The number of new primary cases treated is 5, and 5 more are waiting for the treatment. The number of primary cases in the old series who have been receiving this treatment for quite a long period is 5, of whom one completed 4 full courses, one 5 full courses, two 1 course and one only 3 graftings. None of them have shown any objective improvement. One had a moderate subjective improvement, two had definite subjective (and objective) deterioration, while the remaining two showed no change whatsoever.

Among the 11 secondary cases of the previous year, 2 patients have been treated only with specific treatment, 3 with full courses of tissue therapy along with specific treatment and 2 with partial tissue therapy (i.e. either grafting or injection). One of the above patients had 2 full courses of tissue therapy, but showed no improvement.

Patients have been irregular in their attendance due to various reasons, namely (i) necessity of an attendant (ii) long distance between his residence and the hospital (iii) economic burden of the repeated visits (iv) accommodation difficulties in Calcutta etc.

One of the technical problems that this unit had to face and which could not be solved even after repeated attempts, was the persistently high percentage of protein present in aqueous extracts of placenta used for above therapy. This necessitated the discarding of the two lots of aqueous extracts prepared at an interval of 2 months.

The above two problems associated with the problem of follow up of the patients in the control group considerably hampered the progress of this enquiry. It is now felt that the patients of the control group should be given some treatment for psychological reasons and the duration of tissue therapy reduced to a shorter interval. It is hoped that with these modifications, the above stated difficulties wlll be surmounted.

29. Inquiry into blood stream cooling as a method of inducing and maintaining hypothermia under Dr. A. K. Basu at the S. S. K. M. Hospital, Calcutta

The effect and usefullness of a number of cardioplegic drugs in producing cardiac arrest in hypothermic and normothermic animals were studied.

Altogether 32 experiments have been performed so far.

Method

Adult monkeys weighing between 10 to 15 kg were the test animals. They were anaesthetized with I. V. Pentothal 30 mg/kg body weight, and intratracheal incubation was performed. The intratracheal tube was connected to an automatic respirator, ventilating at the rate of 30-40 cycles per minute. Cooling was effected by the immersion technique and also by rubbing ice on the surface. The range of hypothermia achieved varied from 29°C. to 30°C. Electrocardiographic setup was attached in most of the animals.

Bilateral transternal thoracotomy was performed. The pericardium was extensively opened. The intrapericardial portions of the superior and inferior vena cava were cleared, and the vessels occluded with thick silk thread. After allowing 3 seconds for the heart to empty, the roots of the outflow vessels, viz. the aorta and the pulmonary artery, were occluded by a Pott's clamp introduced through the transverse sinus. The specific cardiolegic drug was injected into the root of the aorta distal to the coronary ostia but proximal to the clamp. In a few cases it was injected into the left ventricle. After the estimated period of arrest, the outflow clamp was released first, followed by the superior vena cava loop. The inferior vena cava loop was released last. For resuscitative measures manual cardiac massage, injections of atropine in some of the acetyl choline cases, injections of calcium salts and in a few cases coronary infusions of 10 per cent sodium lactate solution were used.

Material :

Acetyl Choline Arrest

Comments:—Acetyl choline arrest is immediate and occurs within a few seconds of the injection of the drug. In contrast to potassium induced arrest, the myocardium is not completely flaccid. The duration of the arrest period however, is variable, lasting from a few seconds to 2 minutes. the arrest is often incomplete. The duration of the arrest period could not be increased even after injection of 300 mg of acetyl choline. The incidence of fibrillation during the recovery phase is less striking than in potassium induced arrest, and the survival rate of the animals is higher. Injection of atropine quickly reverts this fibrillatory phase. In normothermic animals with acetyl choline, the indicence of fibrillation during the recovery phase is relatively infrequent.

Potassium Induced Arrest:

Comments :—In potassium induced arrest, the myocardium becomes completely fiaccid and toneless. However, in a number of experiments

there was a variable latent time interval between the injection of the drug and the onset of the arrest phase.

The recovery phase from the arrest stage was always associated with a fibrillatory episode much more marked than with acetyl choline. The release of the inflow occlusion after the arrest period is marked by significant dilatation of the heart. The duration of the arrest period varied from 5 minutes to a maximum of 17 minutes.

Discussion :—From the surgical point of view, potassium induced arrest is advantageous as the flaccid heart of long duration would allow better visualization and facilitate accurate correction of the intracardiac defects. The great disadvantage is the higher incidence of ventricular fibrillation in the recovery phase in almost all the cases.

For the resuscitation of the heart, cardiac massage, if continued for a long time, does revert the abnormal rhythm in many cases. Use of calcium chloride in two cases brought about successful reversion. The effect of sodium lactate infusion in the coronary system was uncertain, and 4 of the 7 animals subjected to this method, died. It has been stated that infusion of oxygenated whole blood in the coronary system would diminish the incidance and severity of the fibrillatory complication therefore, further experiments are being carried out in this direction. Hypothermia is of great value when induced cardiac arrest is brought about by potassium salts. Its value in acetyl choline induced arrest is less certain.

30. Investigation into the pathogenesis of spinal concussion following injuries of spine under Dr. H. K. Sarkar at the S. S. K. M. Hospital, Calcutta.

I. Conditions akin to spinal concussion were produced in rabbits in three stages.

(i) Weakening of the stability of cervical segment of spine by dividing the intervertebral ligaments between spines and laminae from behind in one space.

(ii) After 7 to 10 days acute flexion injury was produced under X-ray control so as to produce immediate complete or partial paralysis of the hind limbs or all the four limbs.

(iii) Animals were killed at varying intervals after 2nd stage by intravenous injection of mag. sulph. Spinal cords were dissected out for histopathological study.

II. *Study of the vascular pattern of the spinal cord in rabbits by injection technique.*

Five experiments were carried out by injection of a dye (1 in 10 solution of Indian ink in buffered formalin) into the root of the aorta under general anesthesia. Three animals died during experiments and in only 2 injection was possible. The roots of the great vessels were immediately clamped after injection after which the animals died. The cervical segments of spine were preserved to study the distribution of the dye in the spinal cord histologically.

Experiments are being continued in this line with other dyes and a reproducible method is yet to be developed.

III. *Study in human cases :—*

A. *Clinical study*—Ten cases of cervical spinal injury were studied from different hospitals. They are classified as follows :

(1) Complete motor and sensory loss—5 cases.

(2) Complete motor loss and sensory escape—3 cases.

(3) Incomplete motor and sensory loss—one case.

(4) Brown-sequard type of lesion, sensory loss on one side and motor loss on the other side—one case.

B. *Pathological study*—Five specimens of the injured portion of the spine along with the spinal cord, of the above-mentioned patients were collected after post-mortem. The distribution of these cases in the clinical groups mentioned above is as shown below :—

Clinical classification		(1) ..Two specimens.
,,	,,	(2) ..Two specimens.
,,	,,	(3) ..Nil
,,	,,	(4) ..One specimen.

The spinal cords showed the following features, macroscopically.

(a) Oedema with fusiform bulging, maximum at the site of dislocation with extension above and below for about 3 c.m. in 3 cases.

(b) Congested blood vessels on surface of the piamater in 3 cases.

31. Hydrocephalus in infents and children-investigation of its etiology and treatment under Dr. R. Nigam at the Medical College, Nagpur.

A. *Experimental.*

1. *Production of hydrocephalus by intrathecal injections of—*

(*a*) *Indian ink.*—Twenty three adult albino rats and five dogs were given either a single injection or repeated injections of a 20 per cent suspension of Indian ink. The immediate mortality was 13 per centin the rats and nil in the dogs. Autopsies were carried out either when the animals died naturally or by sacrificing them at varying intervals. The maximum period or observation was 35 weeks. The findings were as follows :—

(i) Hydrocephalic changes were observed in 50 per cent albino rats and 75 per cent dogs.

(ii) The interval of time since first injection, was in no way related to the degree of hydrocephalic changes observed.

(iii) The ink was found deposited prominently in the basal cisternae, in the cells of the arachnoid and pia. This was mainly perivascular in its distribution. The dura and the brain substance were free from ink. In 50 per cent of the rats and all the dogs the meninges were grossly thickened and adherent. Ventricular dilatation was most prominent in the anterior horns. The hydrocephalus was of the communicating type, the interventricular foramina being widely patent. Rotrograde filling of the ventricles was observed in a few animals. The choroid plexus was not involved.

Histological examination revealed Indian ink deposits in the lining meningeal cells, particularly in the cells along the sulci and the spaces of Virchow-Robin, Atrophy of the cortex and of the basal ganglia was observed along with oedema of the nerve cells.

(b) *Suspensions of Mycobacterium Tuberculosis* were injected into a group of nine sensitised and nine unsensitised albino rats. Only 4 per cent of the animals showed evidence of meningeal irritation with ventricular dilatation. The reactions of meninges were less intense in both groups as compared to those following Indian ink injections. One animal exhibited an arachnoidal cyst formation.

(c) *Suspension of kaolin* was given to 13 albino rats. Immediate mortality was 60 per cent, Hydrocephalic changes wereobserved in only 15 per cent of animals. This substance is not considered suitable for this type of work.

2. *To evaluate the suitability of polythene tubing for shunt operations:*

(*1*) *Peritoneal cavity*-tubing 0.2" diametor was embedded with one end in the peritoneal cavity in four dogs and six

rabbits over a period of three months. The tube was found to be filled with serum and a feeble fibrin clot, and water could be run through the tube freely at a pressure of 60 to 80 min.

(2) *Ureter*—Peolythene tube 0.1" diameter was inserted intc the ureters of seven dogs. No evidence of obstruction was observed in any of the animals.

B. *Clinical*

Fourteen hydrocephalic children were included in the study. The etiological factor was congenital malformation in a majority of cases. Only in one case, laboratory evidence of tuberculous meningitis was observed. Syphilis was not an etiological factor in any case.

The site of obstruction was localised by ventriculography and indigo carmine test. Ten cases were thus investigated. Ventriculography was associated with a mortality of 20 per cent. Four cases were of the communicatiug type and the rest, ventricular type.

Ventriculo-peritoneal shunt was performed in nine cases and the results were as follows :-

1. Good result	(improved symptoms and) (reduction in size of head)	3 cases.
2. Fair result	(improved symptoms with sta-) (tionary head circumference)	3 cases.
3. Failure including operative deaths		3 cases.

Three cases have been followed up, the maximum period of follow-up being seven months.

In the operative technique, a modification found most suitable was to twist in a spiral form, the peritoneal and ventricular ends of the tube prior to insertion. This procedure fixed the tube in position and prevented it from slipping out in the post-operative period.

32. Clinical and experimental studies on keloids under Dr. K. K. Ghosh, at the Medical College, Calcutta.

Up to the end of September, 1958, 74 cases have been subjected to investigation and treatment by different methods. Only the cases of primary keloids were selected irrespective of sex, age and duration for the purpose of this investigation.

Out of the 74 cases, 31 were males and 43 were females. The age incidence was as follows :—

1 to 10 years	2 cases.
11 to 20 ,,	36 ,,
21 to 30 ,,	20 ,,
32 to 40 ,,	13 ,,
to 50 ,,	3 ,,

After filling in the preliminary details according to the proforma submitted last year, the patients were subjected to:

(a) Ultra-violet fluroscopic examination for determination of the nature of blood supply and diffusion of extracellular fluid.

(b) Biopsy—for noting the structural detail.

Basing on the results of ultra-violet fluroscopic examination, the size of the keloid and its duration, tha patients were subjected to different lines of treatment as mentioned below :

Deep x-ray in conjunction with surgery ..	52
Cortisone infiltration alone ..	11
Cortisone infiltration with surgery ..	10
Surgery only ..	41

As soon as the course of treatment was completed, the patients were again subjected to ultra-violet fluroscopy and histopathological examination for noting the changes in the tissue brought about as a result of the treatment.

The cases are now being followed up with particular reference to regression or recurrance.

The details of the treatment.

Group I. Consisting of 52 patients were subjected to Preoperative and post-operative x-ray therapy along with various types of surgical treatment.

Sub-Group : (a) In 34 patients, the keloidal area was subjected to a course of x-ray therapy following which the keloid was removed

and the skin was sutured with least tension using non-irritating suture material e.g, fine silk, nylon and silk worm gut. On completion of wound healing (practically in all by primary intention), the operated area was subjected to a further course of x-ray therapy. In each course 1459 r was administered in 4 days time over a mederate sized keloid.

(b) This sub-group consisting of 7 cases were operated after a course of x-ray therapy and the raw area produced as a result of the removal of the keloid was covered with a skin graft. Following healing of the wounds, the grafted as well as the donor area was treated with x-ray therapy post-operatively with the standard dose.

(c) This group of 8 patients were operated after pre-operative x-ray therapy over the keloidal area and the raw area thereby produced was covered with a skin graft—removed from a previously irradiated donor site on the outer aspect of the thigh. Following healing of the wounds, both the areas i.e. the grafted and the donor were again subjected to a course of x-ray therapy using the standard dosage.

(d) The last sub-group of 5 patients, after pre-oprative x-ray therapy, the keloid was removed in stages from its central part (serial re-section). On completion of the remevel, the area was subjected to x-ray therapy.

Second Group : Consisting of 11 patient only, were subjected to cortisone infiltration alone in the keloid itself as well as in the peri-keloidal zone.

The usual plan of this mode of treatment was weekly or bi-weekly injection of Cortil Acetate (1 c.c. in the keloid and peri-keloidal zone) icc.—25 mg, up to 10 such infiltrations.

Third Grroup : Consisting of 10 patients only, was subjected to local cortisone infiltration following surgical removel of the growth and subsequent suturing of the area.

The plan of this mode of treatment is as follows :

The keloid was excised, the healthy marginal tissue was snbsequently infiltrated with undiluted cortison 1 c.c., and then the skin was sutured. Following removel of the stitches on the tenth day, the infiltrations were made at weekly interval.

Fourth Group : Consisting of one patient alone was subjected to surgical removal of the keloid and subsequent suturing of the area.

OBSERVATIONS :

U.V. Fluoroscopic examination was done in 68 cases. The first evidence of fluorescence appeared within 45 sess. of the completion of injection in faint streaks over the keloid and in the peri-keloidal zone which gradually became denser,

This fluoresence which was noticed in the untrea seen to be grossly interfered in most of the cases treated by different methods.

In the first group of 52 patients treated with x-ray therapy 48 patients were subjected to this investigation.

Out of these 48 patients—only 13 did not show much alteration following treatment with x-ray therapy; and in all these patients recurrence were noticed, though the pre-treatment histological appearances of these cases were no different from those where there were no recurrences. In cases with multiple keloid and in some where the history of trauma was difficult to elicit showed maximum fluorescence in the zones-where the keloids usually occurred, viz, front of the chest, middly portion of the front of the abdomen and the outer and anterior aspect of shoulder joints.

The dimunution of fluorescence was maximum in the peri-keloidal zone and least in the keloidal portion in subjects who responded to this method of examination. In cases treated with cortisone the peri-keloidal fluorescence was not much interfered, the kelold, however, was less fluorescent-

It appears that the vascularity and the extracellular diffusion in the untreated cases is definitely more than the rest of the skin surface and in susceptible cases subjects, the areas mostly affected by this disease shows more vascularity and diffusion.

This diffusion is definitely reduced by x-ray but not much with cortisone infiltration.

OBSERVATIONS ON THE LINES OF TREATMENT.

In the first sub-group of 34 cases, 8 had slight or full recurrence from within 3 to 10 weeks from completion of treatment. In all these cases where there were no recurrence showed broadening of the scar. This broadening was invariable in cases where the keloid was situated over the chese (sternum) or shoulder—suggesting the stress and strain to which these regions are usually subjected. The recurrent keloids were subsequently treated by local cortisone infiltration and all the cases have subsequently become asymptometic but for swelling which has not subsided completely.

The Second sub-group.—Consisting of 7 patients where a skin grafting was done and the donor area was irradiated post-operatively.

Marginal recurrences were noticed in 4 and keloid formation on the donor area were noticed in 6 patients. The recurrent keloid in all these cases were brighly fluorescent.

The third sub-group. of 8 patients where in addition to the usual plan of x-ray therapy with surgery—the donor area was treated just like keloidal one with pre and post-operative irradiation—only 4 showed marginal recurrence in course of five weeks but in none the donor area

exhibited the slightest ten dency of keloid formation though one had multiple keloid on her.

The fourth group of 5 patients—serial resection in the central portion of the keloid exhibited interesting phenomena. The union line never showed any thing beyond a fine incisional scar and keloid formation on the incisional line over the keloid were never noticed. The result of final excision of the keloid cannot be stated at present.

Second group of 11 patients–consisting of incisional keloid following paramedian abdominal incision showed excellent result following infiltration with local acting cortisone. Period and dosage of administration was 1 c.c.—25 mg. of cortisone in the keloid as well as in the peri-keloidal zone—up to 10 such at weekly interval. In all these patients, the keloids subsided completely following the completion of treatment and on an average from 10 to 14 weeks. In two of these keloid subsided completely following the 3rd injection.

In practically all the swelling was asymtometic following the third infiltration. The puncture points on the keloids never gave rise to any form of furher hypertrophy on the keloidal surface. The oldest keloids in these series was less than 6 weeks old.

The third group of ten patients–where cortisons infiltration was supplemented with surgery–the results have been most unsatisfactory and there was complete recurrance in 9 cases in less than 4 weeks time in spite of weekly cortisone infiltration starting from the time of operation.

Fourth group of 1 patient where ordinary surgical removal was resorted showed recurrence in two weeks time in spite of healing by primary intention.

Observations on Histopathologscal Studies.

The normal structural pattern–as stated in the previous report shows the following characters. The covering epidermis resembles the structure of normal skin excepting :–

(a) occasional areas of parakeratosis.

(b) absence of apparadages in the secondary keloids and their presence in lesser number in the primary ones.

(c) The subepidermal layer shows the collagenous connective tissue layer–where there are masses of spindle shaped–fibroblastic cells of various size and thickness, with abundant collagenous matrix, The immediate subepidermal layers shows loosely woven collagenous material. Blood vessels consists of endothelium lined capallaries. Occasional areas of proliferating endothelial cells are also seen in this zone.

Changes after X-rar Therapy.

Clinically regression in the size of the keloid following x-ray has therapy not been seen in any of this series of cases. The histological appearances, however, consists of the following changes :—

1. Minute ulcerations in the epidermis.

2. Prickle cell layer of epidermis contains enlarged cells with granular cytoplasm.

3. In the subepidermal zone, there was oedema and hyalinisation of fibrous tissue which extended deeper. The layer looked more fibrous than cellular.

4. The nuclei of connective tissue showed fragmentation at places.

5. Endothelium lining the blood vessels were oedematous and swollen and contained hardly any corpuscles.

6. Occasional lymphocytic and neutrophillic infiltration.

Cases Treated with Cortisone.

(a) Cases which showed definite clinical regression presented the following picture. The changes were completely in the fibrous tissue layer–where pacchy areas showing brick red collagen consisting of granular amorphous cytoplasm, with scattered nuclear remnant. There were occasional haemorrhagic areas with begining of organisation.

(b) Cases treated with cortisone which showed no clinical regression though asymptometic showed, local collection of round cells (Ch. inflamatory cells) mostly perivascular and scattered all over the field. The collection is suggestive of some kind of foreign body reaction.

The typical histological alteration in cases which showed good clinical response though visible was far lesser in number but the fibrous tissue layer demonstrated generalised loss of compactness. The blood vessels in this fibrous tissue layer did not exhibit any change whatsoever.

Recurrent Cases.

The picture in recurrent cases are of the following type :

(1) The epidermis shows the picture of untreated primary keloid but without any appandages.

(2) The fibrous tissue layer is :

(a) More cellular than fibrous.

(b) The entire layer is much more vascular than the untreated ones.

(c) A typical foreign body reaction was a seen in every case in the immediate subepidermal layer in one a foreign body giant cell was visible in one such centres.

33. Development of biologically specific histo-chemical method for the localization of pituitary-hormones under Dr. V.R. Khanolkar at the Indian Cancer Research Centre, Bombay.

Investigations on the biosynthesis of corticoids, with special reference to the effect of ACTH were carried out. It was observed that corticoidsynthesis takes place in the sheep adrenal homogenates and slices. Conversion of cholesterol to a corticoid-like material has also been shown to take place in the homogenates. *In vitro* stimulating effects of ACTH were seen in the adrenal slices but not in homogenates.

Preliminary work on conversion of progesterone to corticoids in the adrenal homogenate carried out with the intention of isolating a 21-hydroxylating system. It was found that the efficiency of Conversion of progesterone to corticoids was ten per cent.

An intensive staining was noted when sections of ovaries were stained with over new histocheunied methcd for corticoids with ferric chloride followed by Schiff's reagent. This indicated the presence of a corticoid like material in the ovary.

Experiments were carried out to augment the ACTH secreting cells of the pituitary by performing a bilateral adrenalectomy and implanting one adrenal in the spleen. It was noted that the adrenal so implanted remained intact and active and showed hyperplasia in zona fasciculata. There was a moderate increase in the basophil cell population in the anterior lobe of the pituitary gland.

Future Plan of Work

1. Work on the presence of corticoid like material in the ovary will be continued.

2. Biochemical and histochemical studies will be continued in adrenalectomised animals after autotransplantation of adrenals in the spleen.

3. Investigations on the various enzyme systems in the biosynthesis of corticoids will be continued.

34. Survey of malignant lesions of the oropharynx in the population of Guntur District to evaluate predisposing and exciting causes, if any, for the high incidence of these lesions in the area, under Dr. Jagnnadha Reddy at the Guntur Medical College, Guntur.

Methodology.—The research worker, through an "iterview" obtained information regarding age, sex, religion, history of past syphilis, dental condition, oral hygiene, stigmata of vitamin deficiency, particularly of B Complex, analmia and smoking and chewing habits of a 179 cases of histologically confirmed carcinoma mouth and pharynx (126 males and 53 females), (b) 4886 cases suffering from malignant disease other than carcinoma of mouth and pharynx (control) and (c) workers in tobacco factories. V.D.R.L. was done on all patients belonging to the study group.

Carcinoma of mouth and pharynx formed 16.1 per cent of the malignancies registered at the Pathology Department of Guntur Medical College, Guntur.

Cancer of the mouth and pharynx was encountered predominently in the male and to a considerably less extent in the female. This high incidence among the males is perhaps due to the fact that the habit of smoking 'chuttas' (locally made cheroots) is more common among men than women. In the present series of cases suffering from oropharyngeal cancer, 80.2 per eent of men and 45.3 per cedt of women were found to be indulging in regular smoking as against 22.3 per cent in the control series (i.e. groups (b) and (c) above). Beedies were smoked to, only by less than 9.0 per cent of the study group, while cigarette smoking was not popular among them.

Cancer extending from the upper to the lower lip along the contiguous surface at the angle of the mouth was observed in some males who were in the habit of keeping the 'chutta' constantly at this site. Some women had a peculiar habit of smoking 'chuttas' with the burning end turned into the mouth, and among them, surpusingly enough, cancer of the palate was more common. There were no non-smokers among the males suffering from cancer of the floor of the mouth. Thus, a degree of correlation was observed between oropharyngeal cancer and the habit of smoking 'chuttas' in this area. Considerable number of cases of cancer mouth and pharynx in the female and a small number in the male were related to chewing of betel with or without tobacco. The most frequent sites for carcinoma of this type were the buccal mucosa and the gums. This is due to constant keeping of the tobacco quid over these sites.

History of having suffered from syphilis could not be obtained from many of the cases and a very small percentage were positive for V.D.R.L., in the study group. However, among cases suffering from carcinoma tongue 7 out of 40 were positive for V.D.R.L. and hence its causal relationship cannot be over looked.

The association of anaemia with cancer of the tongue in a female of 18 years was noticed. If this is excluded, no form Plummer-Vinson Syndrome was detected in the study group. In 27 cases (15 8 per cent) clinical evidence of vitamin B complex deficiencey was detected in the study group as against 6.2 per cent in the control series.

None in the study group had dentures. The dental state was rather satisfactory except in one case where a projecting tooth was associated with carcinoma of the tongue. Edentia was not noticed in any case. History of consumption of alcohol in the study group and control series was wanting.

Leukoplakia, as a precancerous condition was seen only in 7 cases. This is a negligibly small number compared to 20 to 50 per cent recorded in Pay-Master or in Wynder series.

Conclusions :

1. Carcinoma of the mouth and pharynx forms a high proportion of malignancies in Guntur (16.1 per eent).

2. Oropharyngeal Carcinoma in this area is to a great extent due to 'chutta' smoking and to a limited extent. to chewing of betel with or without tobacco.

35. Studies on the physiology of dermatophytes under Dr. A. N. Chakravorty at the School of Tropical Medicine, Calcutta.

During the current year, alkaline phosphatase, lipase and succinic dehydrogenase activity as well as presence of acid mucopolysaccharides (by Alcian blue method), ascorbic acid, and simple proteins had been demonstrated in some of the species of dermatophytes at various ages of growth of the fungus on different laboratory media.

Histochemical demonstration of alkaline phosphatase, acid phosphatase, lipase, and succinic dehydrogenase in skin sections had been nade in a few cases of ringwor m infection.

Appreciable amounts of alkaline phosphatase were detected biochemically in *T.rubrum* and *M.gypseum*.

Finding of alkaline phosphatase activity by modified technique, and demonstration of lipids, calcium and iron in *T.rubrum* had already been reported last year.

Guinea-pigs inoculated with certain species of dermatophytes, showed no manifest signs over a period of one month. These observations are in progress.

36. Experimental study to investigate antiteratogenic action of (cortisone) on congenital anomalies in rats and mice under Dr. I. P. Agarwal at the G. R. Medical College. Gwalior.

Preliminary observations on 198 foetuses delivered by 33 pregnant rats were available for inclusion in this report. Table sums up the results :-

Table

Groups	No. of animals	Foetuses delivered	Abnormals	
			No. of foetuses	Percentage
Group 'A' (Control)	12	84	2	2.4 per cent
Group 'B' (Trypan blue)	9	30	21	70 per cent
Group 'C'				
C_1 (C/T)	3	12	Nil	Nil
C_2 (C+T)	3	6	Nil	Nil
C_3 (T/C)	3	27	Nil	Nil
Group 'D' (Cortisone)	3	24	3	12 per cent

C/T—trypan blue injection preceded by cortisone (24 hours before).

C+T–trypan blue and cortisone injections at the same time.

T/C—trypan blue injection followed by cortisone after 24 ours.

The tentative conclusions are as follows :-

(a) The incidence of spontaneous congenital abnormalities in laboratory bred albino rats was 2.4 per cent. In both these abnormal foetuses, there was patent ductus arteriosus.

(b) 1 c.c. of 1 per cent trypan blue injected intra-peritoneally in pregnant female rats (7th to 9th day after insemination) successfully induced cardio-vascular abnormalities in 21 out of 30 foetuses, i.e. 70 per cent abnormal foetuses. The induced abnormalities were displacement of auricles, patent ductus arteriosus, patent interauricular septum, common truncus arteriosus.

(c) The subcutaaneous injection of 5 mg. of cortisone acetate either at the same time or 24 hours before or after the injection of trypan blue in pregnant mothers succeeded in completely eliminating the teratogenic action of trypan blue.

37. Enquiry on bacteriophage typing of salmonell typhosa and salmonella paratyphi A under Dr. N. M. Purandare at the Seth G. S. Medical College, Bombay.

A total number of 259 cultures of Salmonella typhosa and 59 cultures of Salmonella paratyphi A were collected from various places, viz., Bombay, Coonoor, Jamshedpur, Madras, Vellore, Amritsar, Ahemdabad, Jerusalem, Tel-A-viv, etc.

In the case of Salmonella typhosa 219 cultures could be typed, 16 were untypable and 6 were typed as degraded strains.

In the case of Salmonella paratyphi A, 51 cultures could be typed; untypable were nil, and 4 were typed as degraded strains.

In the case of Salmonella typhosa, a change in the prevailing types was found. The common types according to their decreasing order were A, E_1, C_4, M, O, and 40. In the case of Salmonella paratyphi A the common types were I and II.

Studies on untypable cultures were carried out according to adaptation method.

38. Enquiry on the role of entamoeba coli as pathogenic organism its under Dr. R. M. Kasliwal at the S. M. S. Medical College, Jaipur.

Twenty experiments were performed on mice, five of which were injected with *E. histolytica* culture, 13 with *E. coli* culture and two with normal saline. It has not been possible to produce lesions in the mice by this injection easily, but lesions could be produced by *F. coli* after incomplete ligation of the colon producing partial obstruction. In one mice *E. coli* has been found penetrating the intestines. Hence *Entamaeba* coli appear to be pathogenic to mice under certain circumstances.

39. Investigations into the role of fungis in pulmonary diseases under Dr. H. S. Andleigh at the S. M. S. Medical College, Jaipur.

Investigations were undertaken to find out the role played by fungi in Pulmonary diseases, and to determine whether the fungus isolated was a primary or a secondary invader. Cases suffering from chronic pulmonary diseases, like bronchiectasis, lung abscess, tuberculosis carcinoma, not showing any beneficial effect by the routine treatment given to them, were selected for investigation. These cases were divided into two groups.

1. Non-tuberculous chronic pulmonary diseases.
2. Pulmonary tuberculosis with sputum positive for A. F. B.

In the first group the sputum was subjected to the following investigations :—

1. (a) Direct examination.

   (i) Four per cent KOH preparation.

   (ii) Gram's staining.

   (iii) Zeihl Neelsen's Staining for A. F. B.

      (*a*) Without concentration.

      (*b*) With concentration.

   (iv) P. A. S. Staining.

   (b) Cultural investigations on the following media.

   (i) Sabouraud's medium with Achromycin.

   (ii) Sabouraud's medium with Achromycin and Actidion.

   (iii) Brain Heart Agar with Achromycin.

   (iv) Brain Heart Agar with Achromycin and Actibione.

   (v) Thioglycollate medium.

Achromycin was added to prevent the growth of bacterial flora and Actidions to check the growth of non-pathogenic fungi.

In the second group, the sputa, in addition to the above investigations, were also cultured on the following media :—

(i) Lowenstein Jensen medium plain.

(ii) Lowenstein Jensen medium with 20 micrograms of streptomycin per millilitre of the medium.

(iii) Lowenstein Jensen medium with 100 micrograms of streptomycin per millilitre of the medium.

Streptomycin was added to the medium to find out if the tubercle bacilli had become resistent to streptomycin which might have been responsible for keeping up the infection.

The following conclusions were drawn :—

1. Out of 32 cases of non-tuberculous chronic pulmonary diseases, 9 cases were positive for fungi. Out of them *C. albicans* was isolated from eight cases and Actinomyces bovis from one case. They were isolated not only from the sputum on repeated cultural examination but also from the bronchial aspirates.

2. Out of the 10 cases of pulmonary tuberculosis, two were positive for *C. albibans.* The acid fast bacilli isolated from these cases were sensitive to streptomycin.

40. Inquiry entitled 'incomplete antibodies and their transmission to the new born babies' under Dr. S. P. Gupta and N. P. Gupta at the K. G. Medical College, Lucknow.

1. *Collection of sera.* —Blood from 50 mothers and from the cords of their babies at the time of delivery was collected with aseptic precautions. Serum was separated and the specimens stored in the deep freeze at—20°C until required.

2. *Determination of the optimal concentration of dextran for detecting incomplete antibodies.*—Experiments were carried out with dextran for determining the optimal concentration. It was found that 4 per cent dextran gave best results.

3. *Each serum has been put in serial doubling dilutions in 4 systems :*

(i) 0.85 per cent saline

(ii) 4 per cent dextran.

(iii) Trypsinised cells in saline,

(iv) Trypsinised red cells in 4 per cent dextran.

So far, 20 pairs of the sera from mother and cord blood have been tested for blood group iso-antibodies.

41. Study of histological changes in the brain in relation to liver injury and blood Ammonia levels in experimental animals and in human cases of liver diseases under Dr. M. Balasubrahmanyan at the Government Medical College, Patiala.

Eight dogs have been used in this study. The normal blood ammonia levels were estimated and ammonia tolerance in these animals determined by the Conway's method as follows :—

The dogs were administered one g. of ammonium chloride dissolved in water through a stomach tube and ammonia determinations were done at half hourly intervals. Normal dogs did not show any appreciable variation in the level of blood ammonia.

Four dogs were administered carbon tetrachloride, by injection twice a week and weekly estimations of blood ammonia are done on these animals. Two animals have since been sacrificed and their liver and brain studied histologically. The blood ammonia levels were slightly raised in those cases where estimations were done 24 hours after the injection.

Attempts were made to produce Eck's fistula in two animals but the experiment had to be abandoned as the animals succumbed in the immediate post-operative period, presumably due to the high temperature. The experiment is being resumed. Attempt is also being made to produce ammonia intoxication by intravenous administration of glycine and to study the acute changes in brain if any as well as changes on repeated ammonia intoxication.

42. Inquiry on changes in brain in liver disease—a clinico-pathological and experimental study under Drs. K. M. Wahal and R. M. L. Mehrotra at the K. G. Medical College, Lucknow.

The purpose of this project is to study morphological variations in the brain in human cases of liver disease, both acute and chronic; and also to observe changes in the brain of animals after producing comparable liver damage.

(a) *Clinical investigation.* —Six cases of liver disease, who died in the hospital, were autopsied during April to September 1958, and the brains and livers were thoroughly studied. All the six cases were of Laennecs cirrhosis of 6 to 38 months duration. All had ascites but none was jaundiced. In three of them coma supervened for one, twenty and twenty one days respectively prior to death. At autopy, livers in all the six cases were moderate to markedly cirrhotic, and in one of them there was superadded hepatic carcinoma. Lobar pneumonia was present as a terminal complicating event in three instances, and fatal haemorrhage following liver biopsy, in one case.

Brains of all these cases presented no gross characteristic features For microscopic studies sections were selected from many representative sites so as to include the cerebral cortex, basal ganglia, thalamus midbrain, pons, medulla and cerebellum. Characteristic changes were, observed more in the cerebral cortex, globus pallidus and thalamus than in other areas. The astroglial changes comprised of marked increase in the size of nuclei with irregularity in the outline and marked hydropic degeneration which made the nuclei extremely pale in appearance. Astroglial processes lost affinity for staining. No particular changes were observed in the ganglion cells, oligodendroglia or in myelin sheaths. These changes were observed only in three cases who had comatose symptoms prior to death, and were not seen in the ramaining three who had no neurological symptoms.

(b) *Experimental study.*—This part of study could be started only in June, 1958 because of delay in the information regarding the sanction of the grant. Albino rats are being utilized in this experiment. Carbon-tetra-chloride was injected repeatedly to produce liver cirrhosis. Within 2½ months cirrhosis of grade I could be produced by the above injections. Sixteen animals have so far been sacrificed. Brains of these animals have been studied from multiple representative sites. No astroglial changes comparable to those found in human cases were observed. This is not surprising, because even in human cases changes were not observed unless neurological symptoms supervened. The other animals in the series are under observation. They will be sacrificed when cirrhosis progresses to grade III.

43. Enquiry on the osmotic tonicity of the cells of gastric mucous membrane and its regulation under different conditions under Dr. K. C. Basu Mallik at the N. S. Medical College, Calcutta.

The osmotic tonicity of the cells of the gastric mucous membrane was determined indirectly by immersing pieces of mucosa in salt solutions of different osmotic concentration and estimating their water content. The concentration at which there was neither gain nor loss of moisture was assumed to be isotonic with the cells of the gastric mucous membrane. This was found to be higher than the concentration of salts in the extracellular fluid. It was, therefore, assumed that to maintain this high osmotic tonicity the cells have to spend energy which is obviously derived from respiration. So the oxygen uptake of pieces of mucosa immersed in salt solutions of different concentrations was measured and their water content was determined after the end of the experiments. It was observed that respiring slices resisted the variation of moisture content much more vigorously than the slices kept in cold saline of similar concentration. But it was realised in course of these experiments that the whole variation in water content could not be explained on the basis of osmosis as changes due to hydration of colloid were also taking place. This aspect of the problem needs further investigation which is proposed to be undertaken in future. Another interesting feature observed was that the cells of the gastric mucosa immersed in salt solutions of different concentrations showed only slight variation of oxygen uptake which suggested that in spite of the alteration of osmotic concentration of the fluid to which the cells were exposed the metabolism of these cells was not grossly influenced, This is perhaps not very surprising when one recalls that of all the different tissues of the body, it is the gastric mucosal cells which have to face the markedly altered osmotic concentrations in the fluid in which they remain in contact for a prolonged period. Thus when a person drinks only pure water the osmotic concentration of the gastric juice falls very low while, when he drinks thick syrupy fluid, the osmotic tonicity is raised very much. In spite of the wide range of variation in the osmotic concentration of the intragastric fluid the mucosal cells do not show much functional change. This aspect of the gastric function has been elucidated by the oxygen uptake experiments.

44. Experimental Production of Cancer in Mice with Tobacco Tar and Heat under Dr. D. Govinda Reddy at the Andhra Medical College, Visakhapatnam.

A high frequency of palatal cancer is recorded in women who smoke cigars with the burning end inside the mouth in Visakhapatnam District and surrounding areas; whereas in men who smoke the same kind of cigars with burning end autside, the incidence of palatal cancer is low. Since the thermal factor is the only difference between the two sexes, to account for the decidedly preponderant occurrence of palatal cancer in women, experiments were undertaken to evaluate the role of heat in the production of cancer.

Tobacco-tar was prepared by condensation of smoke from burning cigars in ice-cooled acetone. White rats as well as mice were used for the experiment. The animals were divided into three batches; one batch was painted with tobacco-tar, another exposed to heat only, and the third to both tobacoo-tar and heat at the same site. Animals exposed to heat showed degenerative changes in the epidermis and dermis. From among the animals exposed to tar only, while rats failed to show any tendency for neoplastic changes, but in the case of white mice there was definite evidence of hyperplasia of the epidermal epithelium. The skin of mice exposed to tobacco-tar and heat showed papillomatous elevations of the skin and histological evidence of malignant transformation of the cells of the epidermis and sebaceous glands. By painting with cigarette-tar Wynder *et al.* produced cancer in 44 per cent of the mice used (900), that only after nine months. In the first stage of the present experiment, cancerous changes were found even before three months. So heat seems to accelerate neoplastic changes.

5. Serological studies on tropical eosinophilia under Dr. B. P. Saxena at the G. R. Medical College, Gwalior.

This work has been started only in August 1958. Complement titres estimated on 10 normal subjects within the last 4 months gave results comparable to those obtained on a group of 100 normal persons previously. The results showed that in normal sera, a dilution ranging from 1:4 to 1:8 could bring about 100 per cent haemolysis. However, if 50 per cent haemolysis is taken as the end point, the range of serum dilution varied from 1:16 to 1:32.

Estimation of complement titres was carried out in the sera obtained from 30 cases of tropical eosinophilia in the last four months. Earlier 115 cases of tropical eosinophlia were also subjected to similar investigations. These results indicated that in a majority of cases suffering from tropical eosinophilia, requiring sometimes neat undiluted serum to bring about 100 per cent haemolysis.

During the above period a coloremetric method for the estimation of serum emplement has been developed taking 50 per cent haemolysis as the end point, this method is found to be a definite improvement over the naked eye method taking 100 per cent haemolysis as the end point.

46. Experimental studies in the production of hypersensitivity in animals under different conditions of endocrine metabolism under Dr. D.N. Shivpuri at the Vallabhbhai Patel Chest Institute, Delhi.

A. *Preliminary Efforts at Sensitizing the Guinea-Pigs :*

Prior to the main experiments, attempts were made to sensitize the guinea-pigs by different antigens in different doses.

B. *Adrenalectomies Performed.*

With the object of standardizing operative techniques as well as pre-operative and post-operative care, bilateral adrenalectomy operations were first carried out on dogs, and later on rats and guinea-pigs. The operations were done on 3 dogs, 6 rats and 9 guinea-pigs, in one stage. A single incision was adequate in all animals except one dog.

C. *Sensitization Experiments in Different Groups of Animals.*

These experiments are performed on dogs, rats and guinea-pigs, each divided into several groups. One group of animals is sensitized but not adrenalectomized, another group is adrenalectomized and then sensitized, but receives substitution therapy with DOCA and cortisone. The third group is the same as previous one, but receives no substitution therapy, after one week. Last group is the control one, i.e., non-adrenalectomized and receiving no sensitizing injection.

After three weeks incubation period, all the animals will be examined for the degree of hypersensitivity or any symptom of asthma by the following tests :—

(1) Skin test (2) Precipitin test for assaying the anti-body-titre (3) Shock-dose by intravenous or intra-cardiac injection.

47. Enquiry on the natural history of nephritis an experimental study under Dr. K. P. Sengupta and Dr. B. K. Aikat at the Institute of Post-Gradute Medical Education and Research, Calcutta.

A. *By organ specific antibody :*

(i) Preparation of kidney antigen and of anti-kidney sera.

The kidneys were removed asceptically from albino rats following perfusion *in situ* with sterile 0.85 Nacl. A 20 per cent suspension of kidney homogenised in 0.85 per cent Nacl was prepared and injected into a total of 16 rabbits, each weighing 2-2.5 kg. intraperitoneally 3 times a week for 3 weeks. After rest of 2 wks. they were bled by cardiac puncture. Sera were pooled and inactivated at 56°C for ½ hour just prior to injection. Each of the sera was tested for the presence of antibody by the technique of Feldman and Yaffe (*Nature, 179*, 1353, 1957).

(ii) White albino rats of either sex maintained on 'balanced' Conoor diet were weighed 110g$\pm$10 g. Injection were made intraperitoneally or intravenously either a single injection or five consecutive days with pooled sera.

(iii) Test to determine the course of nephritis.

Urine samples were collected after putting animals in metabolic cages daily for a week before injection and also daily after injection for another week. Thereafter, the urine specimens were collected weekly, or monthly until animals were killed.

Urinary proteins were determined by Savisky-Stafford technique. Rats were inspected for oedema and weighed daily. Blood urea and N.P.N. and serum cholestrol was determined on blood drawn at necropsy. Rats were killed by stunning on the head and kidneys were fixed in cold buffered formalin, cold acetone and buffered formalin. Paraffin section from kidneys are studied by Haematoxyline and Eosin, PAS and Alcian blue, PAS and Mallory's trichrome. Frozen sections were stained in Sudan III and IV and also routinely for alkaline phosphatase.

B. By injection of Nephritogenic strain of type 12-gr. A. Streptoccocci.

Total 4 groups were studied in 3 groups of animals of 5 each were studied all these were adult of either sex weighing 2.25 kg. In one groups of small animals weighing between 400 and 500g were used.

Type 12 strain of gr. A streptoccocci was obtained through the courtesy of Collindale Laboratories, London. A 24 hours growth in blood agar was taken in saline suspension and density of suspension was measured by Brown's opacity tube. A constant density was used every time and injected subcutaneously into rabbits after shaving their backs after an interval of 15 days for 4-5 months. After the last injection a challenging dose of the same organisms was given intramuscularly. Animals were killed after an intervals of 2 wks. 4 wks, 6 wks and8 wks. and 4 months. Estimations of blood urea and N.P.N. and urinary. Protein were done during the time of killing.

Results

(A). In rats injected with anti-rat kidney rabbit sera (ARKRS).

1. Course of nephritis.

Elevation of blood urea, N.P.N. and urinary proteins, and renal histopathology presented the definitive presence of nephrits. When injected with ARKRS initiated an immediate nephritis but the course appeared to depend on the dosage.

2. Renal pathology.

(A) Rats given ARKRS exhibited renal lesions which, in most instances, paralleled the manifestations of nephritis as evidenced by proteinurea, elevation of blood urea and N.P.N. Autopies were performed at first daily for 1-8 days, then-bi weekly, or monthly. Earliest lesions was seen on the second day onwards in the glomeruli in the form of oedema and hazziness of basement membrane. There is increased in the number of cells of glomerular tuft. The increased cellularity of glomerulus, unfolding of the tuft, gradual diminishing of blood spaces and later filling of the whole glomeruli. In some mitotic figure could be demonstrated. Adhesion to the capsule in proliferating glomeruli was observed earliest in second week onwards. In latest stages by hyalinasation of the glomeruli and round cell infiltration were also seen. The tubules show degenerative lesions in the form of vacuolation, hyaline droplets and contain in many of them eosinophilic tubular cast. Tubular regeneration was, however, not observed.

(B). Naphritic lesions: *Produced in the rabbits by injection of Streptoccocci : Gr. A type 12 strain*. Proliferative glomerular lesions were observed in the early stages and in those cases which were followed up to 4 months after the last injection, showed evidence of chronic glomerular nephritis.

48. Studies on biochemical and clinical aspects of leucoderma under Dr. B. Banerjee at the Medical College, Calcutta.

Definite colitis due to one or more specific intestinal infection (other than bacterial), is present in 15 out of 22 cases of leucoderma under investigation in the present series. The remaining 7 are free from clinical manifestations of colitis. Trauma and irritation (due to Cosmetics, Drugs etc.) are additional factors in a few cases. Treatment of specific intestinal infection and locally, pigment stimulating applications resulted in absolute response in 2, and partial or satisfactory response in 9 in the former and 5 in the latter group 4 in the former and 2 in the latter group have shown poor response. Response is not satisfactory in melung, mecocutaneous, cosmetic types and in late cases. Generalised type with shorter duration or those appearing shortly after gastro-intestinal attack responded favourably,

Biochemical examination of all these patients has shown defects of tyrosine metabolism. All cases showed increase of tyrosine in blood. Definite improvement was noticed in all cases with high protein diet in addition to specific treatment for colitis and pigment stimulation.

Biochemical—Ten different samples of blood plasma from patients admitted to the hospital have been analysed for free and protein amine acids by the paper chromatographic technique.

The lipid content of whole blood from both normals ond leucoderma patients was studied. The total lipid was further fractionated into phospholipids (lecithin, cephalin), and cholesterol. The significance of these data will be assessed when the investigations are completed on a large number of cases.

49. Effects of partial obstruction of bile ducts on secretion and composition of bile and structure and function of liver in dogs, under Dr. J. D. Sachdev at the M. G. M. Medical College, Indore.

A group of five dogs were subjected to partial obstruction of common bile duct under aseptic conditions by using the method of Duchesne *et al* under either anaesthesia. Total plasma proteins, alkaline phosphatase, thymol turbidity, icteric Index, Vandenberg test and prothrombine time were estimated to assess the liver function in these animals before operation and after seven and fourteen days of partial obstruction of the common bile duct.

No appreciable changes in the values were observed on the third day of obstruction. Values after 7 and 14 days indicated a progressive liver damage. An attempt was made to correlate these results with biopsy findings.

50. Studies to determine the role of vitamin C, on the healing of fractures under Dr. K. N. Udupa, Civil Surgeon, H.P. Hospital, Simla.

The main purpose of carryiug out this study is to find out whether the period of healing fractures can be shortened by administering vitamin C.

Sixteen guinea pigs were divided into two groups, group A comprising of 4 animals on a scorbutic diet, being fed only upon bran and water, group B comprised of 12 animals on a normal diet consisting of bran, gram, fresh green leafy vegetables, catmeal, linseed, taramira and salt.

The left femurs of all the animals were fractured under general aether anaesthesia.

The animals in group B were further divided into sub-groups. Animals in group B 1 were continued on the normal diet as stated above.

Animals in group B 2 were given the normal diet and in addition were given I M injections of vitamin C in a dosage of 50 mg. per kilogram of body-weight, three times a week.

Animals in group B 3 were continued on the normal diet and in addition were given 25 mg. of vitamin C at the site of fracture.

Animals in group A, however, were continued on a scorbutic diet. All fractures were X-rayed on the same day or the day following the fractures.

No treatment was given to these animals for a week after which they were treated as stated above.

51. An experimental and clinical evaluation of synthetic substitutes for autogenous fascia in muscle transference operations around the hip, under Dr. S. K. Chatterjee at the Nilratan Sircar Medical College, Calcutta.

Procedure—The animals were divided into five groups. In one group, under anaesthesia, the upper end of a strip of autogenous lumbar fascia was sutured to the lower end of the detached lateral part of the sacrospinalis muscle and the lower end of the strip to the periosteum of the femur near the gluteal insertion keeping the limb extended, and this was immobilised in plaster of paris casing. In the other groups, a strip of nylon mesh or terylene cloth either naked or encased in polyethylene replaced the autogenous fascia in the above operation.

Observation :—After the conclusion of a pilot experiment on a group of rats the main experiment has been undertaken on rabbits. The following studies are being carried out:—

(a) Bacteriological culture of the wound.

(b) Power of the transferred muscle to move the hip.

(c) The reactions (macroscopic and microscopic) to fascia and prostheses.

(d) Estimation of tensile strength of fascia and prostheses and the junctional areas.

52. Determination of the relation between intra-ocular tension and the hyaluronic acid hyaluronidase enzyme system, under Dr. K. R. Kesavachar at the medical College, Nagpur.

The scheme of this research had to be changed as more recent references regarding estimation of hyaluronic acid were obtained. According to these references 94 per cent of the Hyaluronic acid present in the aqueous is in a depolymerised form. Hyaluronic acid, as such, occurs in such a minute amount that unless a large quantity cf the acqueous is obtained the substance cannot be estimated.

Hyaluronic acid comprises of equal parts of glucuronic acid and glucosamine. It was this glucosamine that was estimated after hydrolysing the hyaluronic acid in the aqueous with hydrochloric acid. Estimation of glucosamine was done bv utilising the Elson Morgan reaction. The aqueous contains very negligible quantity of proteins. There is no other substance in the aqueous which responds to the Elson Morgan reaction. The amount of hyaluronic acid is expressed in terms of glucosamine in micrograms per gram of aqueous. The average amount of glucosamine in 5 samples reported by Mayer Smythe and Gallardo was 16 micrograms per gram. The samples of aqueous used here showed an average glucosamine content of 12 micrograms per gram. Unhydrolysed aqueous was also tested as a check, but it did not develop any colour with the Elson Morgan reaction.

53. Circulation of aqueous of humour—its study by fluorescein technique and radioactive tracer substances sodium 24 (crystalloid) and iodine (colloid) under Dr. Sen Gupta at the Medical College, Calcutta.

Circulation of aqueous humour in human eyes was first studied by fluorescein technique. Two c.c. of 10 per cent sodium fluorescein solution was administered to the patient intravenously. Specific ultraviolet band using Chance Bros filter Ox-I with Tungsten lamp of Carl Zeiss Slit Lamp apparatus was allowed to fall on the eye when fluorescence was observed in the aqueous humour of anterior chamber. The measurement of fluorescence was made at 5 minutes interval by using selinium photocell connected to a highly sensitive moving coil galvanometer with lamp and scale arrangement. The decay of fluorescence with time was noted for half an hour. Deflections, however, though responded to expectations were restricted to a short travel.

An attempt was also made to use the photographic method for measuring fluorescence. The fluorescent eye was photographed with contax camera with 2.8 Tessar lens successively for half an hour at an interval of 5 minutes. The negative of these photographs were put to Zeiss Extinction marker II to measure the extent of black deposits of the negative print, *i.e.*, luminous density of fluorescence in some arbitorary scale. The extinction marker showed the variation of deposite on the print graphically but the result in our opinion is not decisive.

However, the observations with photocell and moving coil galvanometer method unfolded tremendous possibilities for the success of the method. In persuation of this method I am endeavouring to secure a more sensitive photocell *i.e.* Caesium cell for detection of very minute change in fluorescence coupled with an electronic amplifier for significant and larger deflections.

Circulation of aqueous humour was next studied by tracer technique. Radio-iodinated (131) human serum albumen (RISHA) was injected intravenously (Dose, 50 micro curies) and the uptake in the aqueous humour of the Radio-active material was measured using Collimated Scintillation probe. Clearance pattern was studied by injecting the same radioisotope (RISHA) into the anterior chamber (Dose, 0.25 micro-curie). Subjects were all human beings of old and adult age groups.

The results of these investigations showed that in old age groups the uptake of the radioactive colloidal material is more flat in character as compared with the same in theadults. The rate of inflow is slow with that of outflow remaining more of less the same, and this state of affairs becomes more conspicuous with the advancing age. This amount to stagnation of the intraocular fluid to some extent, and this means that the intraocular fluid will lose much of its food materials ; on the other hand. it will become loaded more with the metabolic products of the cells. The clearance of the colloidal material was studied only in a single case and so no definite conclusion can be drawn as yet. Even this single case showed that rate of clearance is slower than that of inflow. It is important to note that all the above studies have been made only with colloidal particles.

54. Investigations of hearing in the deaf by means of speech audiometer with particular view to evolve a set of words in Hindi under Dr. R.N. Misra and Dr. M.L. Bhatia at the K.G Medical College Lucknow.

Fifty normal persons and seven deaf patients have been tested by means of the speech-audiometer, using the English word list, prepared by the Psycho-Acoustic Laboratory, Harvard University, as test material.

Results of these tests reveal that in normal persons the discrimination score of 100 per cent cannot be obtained in most of the cases when using English words as test material. The zero-level also does not correspond to the zero-level of the English people in 15 cases. The results of these testings are not yet conclusive, as the same normal persons have to be re-tested with the Hindi test material and the results compared before definite speech levels can be determined in the results of deaf patients are also likewise expected to show difference when they are tested with Hindi test material.

A list of 90 test words in Hindi (disyllables) has been prepared. These words will be subjected to a thorough standardisation and clibration at the All-India Radio Studios, Lucknow, before permanently recording them on gramophone records on Vinylite pressings.

55. Studies on glucose content of skin and blood in patients with some infections of skin under Dr. T. D. Majumdar at the Nilratan Sircar Medical College, Calcutta in collaboration with Dr. B. Chakarbarti, Asst. Prof. of Physiology of the same institution.

Patients suffering from a particular variety of follicular infection of the skin which is supposed to be "Skin-diabetes" formed subjects of the study. Since the work has commenced only in the last week of July 1950 it has been possible to analyse the glucose content of skin and blood of only 15 cases of the above type. The method adopted for the estimation of glucose was that of Hagedorn-Jenson with appropriate modification for estimation on the skin. The results showed that on almost all these cases, glucose content of the skin was altered suggesting their possible relationship to the clinical condition of "skin-dibetes". Along with this work, investigations have also been carried out on 61 cases, to find out the susceptibility of skin of various infections to the disappearance of injected normal saline solcution subcutaneously at the site of infections.

56. Study of distribution of atherosclerotic heart disease in various social groups in Delhi and its correlation with serum cholesterol and lipid levels under Dr. S. Padmavati at the Lady Hardinge Medical College, New Delhi.

This enquiry has been in progress since 1-4-1955. For the first time in India, an attempt has beən made to find out the incidence of various types of heart diseases in population groups with reference to their dietary patterns and blood lipid levels. So far 251 persons form high income groups and 1478 persons from low income groups (comprising industrial labourers, rural and domestic workers etc.,) have been studied.

I. Heart Disease in Various Population Groups

1. *Incidence of heart disease.*—The figures on the incidence of heart disease in India related only to hospital statistics from a restricted group of only 5 states. The present venture of studying the incidence in various population groups has brought out the fact that Rheumatic heart disease and Cor-pulmonale are more prevalent in the rural population, whereas Coronary heart disease and Hypertension are more common in the high income classes concentrated in the urban areas.

2. *Prevalence of E.K.G. abnormalities.*—The prevalence of non-specific E.K.G. abnormalities in various population groups has been investigated. In the light of work abroad, Ekh abnormalities should be compared in different population groups rather than interpreted for single groups The incidence of such abnormalities in high and low income groups is significantlly different, being 4.6 per cent in the former and 2.7 per cent in the latter.

Blood pressure levels and hypertension.—It has been possible to establish average blood pressure levels in the various age groups in the high and low income classes. These figures from Delhi are the first published work of their kind from India. The differences between high and low income groups have been ascribed to differences in body-weight in the two classes.

The incidence of hypertension is much lower in Indians of both income groups when compared to Western countries.

II. Dietary Patterns In Different Social Groups :

The dietary intake patterns of different social groups have been worked out to form a base line for such surveys in future, It has been possible to compare the present dietary survey with one made 20 years ago in the same groups in Delhi. The diets seem to have deteriorated during this period.

III. Blood Lipid Levels

Blood lipid levels in high and low income groups have been worked out. The Serum Cholesterol levels are considerably higher in the high

income groups than in the low income groups at every level of age, body weight, fat intake and physical activity. The figures in these two groups are comparable to those obtained by Keys and others on similar social groups in Western countries.

IV. Lipoprotein Patterns

The work on this aspect of the problem has just been started and so far about 120 sera have been studied.

57. Study of environmental and nutritional factors affecting the incidence of atherosclerosis and coronary heart disease under Dr. K. S. Mathur and Prof. P. N. Wahi at the Medical College, Agra.

1. A total number of 102 cases of coronary heart disease and 100 cases of essential hypertension have been studied. The various environmental and nutritional factors operating in them have been evaluated.

2. 817 persons from general papulation have been surveyed to find out the incidence of coronany heart diesease. They have been classified into five socio-economic groups (a) professionals executives and big businessmen-188, (b) Clerical and allied workers-241, (c)Industrial workers-228, (d) Manual workers-112, and (e) Agricultural workers-48.

In addition to eliciting a detailed medical and dietetic history, a thorough cardiovascular check-up has been done in all cases. Lipid studies, electrocardiography and other relevant laboratory investigations have also been carried out.

3. The professional group showed a peak incidence of coronary heart disease (3.7 per cent). The incidence in clerical and allied workers representing middle class society, was only 0.81 per cent. No case of coronary heart disease was encountered in the industrial, manual and agricultural workers.

Incidence of hypertension in the professional group was 6.4 per cent, in the clerical group 4.1 per cent and in industrial workers, 1.7 per cent Manual and agricultural workers were free from hypertension.

4. The dietary analysis of different socio-economic groups revealed that professionals who showed greater incidence of coronary heart disease consumed the largest quantities of fat (77.1 ± 25.8 Gm.) supplying 32.8 ± 9.4 per cent of the colories in the diet.

5. In the lipid studies, total serum cholesterol, free cholesterol ester cholesterol and lipid phosphorus have been estimated. The relationship of dietary cholesterol and dietary fat to blood lipids has been worked out. The levels of blood cholesterol at different age groups have been stuided.

6. Effects of different Indian dietary fats on blood coagulability and serum lipids have been studied in cases of coronary heart disease and normals. A significant reduction of coagulation and prothrombin time has been found after a fatty meal. The extent of reduction in normals and in cases of coronary heart disease, however, was not much different. The effects of different dietary fats are also being statistically analysed.

58. Study of the pattern of coronary circulation by injection technique with special reference to its relationship to the incidence of coronary heart disease, under Prof. P. N. Wahi and Dr. K. S. Mathur at the Medical College, Agra,

During the year, the assignment was to cotinue the work under the enquiry, along with a study of the incidence of atherosclerosis in medicolegal and other autopsies. The latter part of the assignment could not be fully implemented as the clinical data obtained on the autopsy cases was very inadequate. In addition, in a majority of the cases the material was received in an advanced stage of post-mortem autolysis, where no adequate study could be carried out.

In continuation of the study carried out previously, eleven more hearts have been injected, dissected, x-rayed and studied by the modified Schlesinger's technique, thus bringing the total number studied by this technique to 42.

In addition, 20 hearts were also studied by Miale and Bledsoe's technique.

CASES STUDIED BY MODIFIED SCHLESINGER'S TECHNIQUE

Eleven hearts studied by this technique belonged to age groups ranging from 15 to 70 years. Out of these eleven cases, seven were from male and four from female subjects. The weight of these hearts varied from 200 to 270 grams. All these hearts were obtained from medicolegal cases brought for autopsy, hence no history pertaining to corlonary disease was available.

Out of these eleven, nine belonged to type I heart (right coronary preponderant) and two to type II (balanced circulation type).

A gross study of these hearts revealed slight degree of atherosclerotic change in only one, seen in the proximal 1.5 cm of the left coronary artery. No evidence of any anastomosis was seen between the two coronary arteries.

MIALE AND BLEDSOE'S TECHNIQUE

Twenty human hearts were studied by this technique. This is a combined injection-dissection cross-section technique designed to (a) demonstrate the normal and abnormal patterns of coronary circulation (b) determine accurately the blood supply of the muscle bundles, and (c) support these observations by a study of the distribution of myocardial damage due to coronary narrowing or occlusion.

The technique combines the advantage which an injection technique offers in studying the state of the coronary arteries with data referable to muscle bundles, which can only be obtained from the study of cross-section slices. The technique is simple, and has been found to be equally good. Here radio-epaque barium mixture is used as the injection mass. A skiagram is taken of the uncut heart, and also of the cross-sectioned slices after 7 days fixation in 10 per cent formed saline. The pattern of the blood supply as studied in these cases is in agreement with Miale and Bledsoe's work.

As the present study did not include any case of infarction, further evaluation of the technique was not possible.

59. Enquiry on the role of (a) adrenal cortex, (b) stress and (c) cholesltreol in the pathogenesis of atherosclerosis under Dr. (Mrs.) S. Sachdev at the Medical College, Indore.

White leg horn cockerels, 15 weeks, old were divided into three groups.

Group—A. Normal birds on synthetic diet.
Group—B. Birds on synthetic diet containing 1 per cent cholesterol.
Group—C. Birds on synthetic diet containing 1 per cent cholesterol and subjected to stress by injecting (a) formation and (b) adrenaline.

The values for serum cholesterol at the time of sacrifice in group A ranged from 130 to 173 mg. per cent with an average of 148.3 mg. per cent. In group B the values ranged from 169 to 291 mg. per cent with an average of 240 mg. per cent. In group C animals subjected to stress by formalin injection, serum cholesterol values ranged from 160 to 273 mg. per cent with an average value of 213 mg. per cent, while in animals given adrenaline, the values ranged 230 to 327 mg, per cent with an average value of 260 mg. per cent, There is thus, a rise in serum cholesterol by as much as 62 per cent in group B and 75 per cent in group C when compared to those in group A.

Macroscopic examination of the heart and blood vessels revealed atherosclerotic lession in 75 per cent of group B and 71 per cent of the group C animals. Lesions were mostly in the form of fatty streaks and in some atheromatous ulcers were seen. Macroscopic findings were corroborated by microscopic studies. These results, however, were not in line with our previous findings with regard to the part played by stress in athero- sclerosis.

60. Pulmonary hypertension under Dr. K.K. Datey at the Seth G.S. Medical College, Bombay.

With the advent cf cardiac catheterisation, the study of hemodynamics in various heart diseases has become possible. As our ideas regarding the therapy of a cardiac patient are now oriented in terms of hemodynamics, it has become a necessity to devise ways and means short of cardiac catheterisation, to judge the pressure in the lesser circulation. With this in view, this study was undertaken to correlate precisely the pressure changes in pulmonary circulation with clinical findings and electrocardiographic changes.

These investigations were carried out on 35 cases of pulmonary hypertension. Cases with P.A. systolic pressure of 30 mm. Hg. or more were included in this series. The etiological breakdown of the cases was—mitral stenosis (9 cases), mitral stenosis with minimal aortic leak (3 cases), mitral stenosis with mitral incompetence and tricuspid stenosis (one each), 3 cases each of patent ductus arteriosus and ventricular septal defect, 5 cases of atrial septal defect, primary pulmonary hyperten sion (4 cases) and one each of Lutembacher's syndrome, anomalous pu lmonary venous drainage, chronic corpulmonale, constrictive peric arditis and aortic incompetence. One case had persistent ductus an d ventricular septal defect. Each case was clinically evatluated. Investigations including an electrocardiogram, a teleradiogram and right heart catheterisation were carried out in every case.

A. *Correlation of pulmonary resistance to pulmonary hypertension :*

In general, it was found that the rise in P.A. pressure roughly paralleled the increase in pulmonary resistance, in cases of post-capillary hypertension. In cases of left to right shynt, there was a greater rise in pulmonary artery pressure as compared to that of pulmonary resistance, probably beoause of the additional factor of increased flow.

B. *Correlation of pulmonary hypertension with clinical findings :*

The usual clinical findings, due to pulmonary hypertension, perse, in this series, were prominent 'a' waves in the jugular venous pulse (unless complicated by tricuspid incompetence or auricular fibrillation), right ventricular heave, systolic ejection click or murmur in pulmonary area and accentuation of the pulmonary component of the second sound. A preliminary analysis of the clinical data have brought out the following points :—

(i) The systolic ejection click was heard in most of the cases. Right ventricular heave was felt in all cases, except in cor pulmonale and constrictive pericarditis, where it was masked by emphysematous lung and pericardial constriction, respectively. It was noted that in general the intensity of the heave increased with the rise in pulmonary pressure.

(ii) Pulmonary incompetence: This was detected in seven cases. In six, the pulmonary artery pressure was more than 90 mm. of Hg.

However, four other cases with pressures more than 90 mm. of Hg. did not show pulmonary incompetence.

(iii) Signs of tricuspid incompetence were present in ten case, In 9 of them the P.A. pressure was 80 mm. of Hg. or more.

(iv) 'a' wave in right atrium : The pulmonary artery pressure was normal when 'a' wave was less than 5 mm. of Hg. (above the auricular level); with a few exceptions the 'a' wave was between 5-15 mm. of Hg. when the P.A. pressure was between 40 and 80 mm. of Hg. When 'a' wave was more than 15 mm. of Hg., P.A. pressure was invarably more than 80mm. of Hg. in the absence of tricuspid stenosis and constrictive pericarditis.

C. *Correlation of electrocardiographic findings with the hemodynamic data :*

The electrocardiograms were analysed and the pulmonary artery systolic pressures were correlated with the height of P wave in standard limb lead II, the axis of P wave in the frontal plane, the height of R wave, R/S ratio, the time of inscription of intrinsicoid deflection in V_4R and V_1, the appearance of qR pattern or its modifications in right precordial leads, and R/Q ratio in aVR.

Auriculogram

(1) Voltage of P wave in standard limb lead II : In this series, the height of P was 2.5 mm. or less in 21 cases, in spite of pulmonary hypertension. In some cases, voltage of P was normal even when P.A. pressure was markedly elevated. P wave of high voltage was, however highly suggestive of pulmonary hypertension. Patients with P wave 3 mm. or more in height, in the absence of tricuspid stenosis had usually severe degree of pulmonary hypertension. Although, in general, the taller the P wave, the greater was the P.A. pressure, there was no linear relationship between the two.

(2) Axis of P wave in the frontal plane : The axis of P, as measured by amplitude ranged from +30 to +90. In the series of 14 cases of mitral stenosis the axis of P was less than +45 only in one. This is because, all the cases had pulmonary hypertension which usually resulted in a shift of P axis to the right.

Ventriculogram

(1) Among the acquired heart diseases, R.V. hypertrophy was seen in a case of mitral stenosis even at a P.A. systolic pressure of 40 mm. of Hg. Five cases with pulmonary hypertension did not show R.V. hypertrophy pattern at all, because of the concomitant left ventricular enlargement.

(2) Axis of QRS in the frontal plane : In cases without left ventricular hypertrophy, the axis of QRS in the frontal plane was more than 100 in all except 2 instances. In these 2 cases, the P.A. systolic pressure was 30 and 40 mm. of Hg., respectively. In the presence of L.V. enlarge-

ment, the axis shifted from left to right with the development of pulmonary hypertension, but was still less than 100 in this series. There was no linear correlation between the shift of axis to the right and the degree of pulmonary hypertension.

(3) Right precordial leads (V_4R and V_1)

(i) Patterns: The usual patterns seen were Rs, rS, qR or its modifications, rsR' and R. Excluding one case each of constrictive pericarditis and chronic cor pulmonale, there were 14 other cases which showed Q wave in right precordial leads. All these cases except one, had P.A. pressure more than 60 mm. of Hg., and in 9 cases it was more than 80 mm. of Hg. rsR pattern was seen in 5 cases associated with A.S.D., one case of anomalous pulmonary venous drainage and 2 cases of mitral stenosis. The P. A. pressure ranged from 30-95 mm. of Hg.

(ii) R/S ratio: There was no correlation betweed R/S ratio and P. A. pressure.

(iii) Height of R wave: In case of pure right ventricular hypertrophy, the average P. A. pressure was 62 (30-110), when the height of R was less than 5 mm. It was 85 (50-115) and 116 (48-135) mm. of Hg. when R wave was between 5-10 mm. or more than 10 mm. respectively.

(iv) Time of inscription of intrinsicoid deflection: The cases were divided into 2 groups:

(a) Those showing rsR' pattern. In this group, no correlation could be made out with degree of hypertension.

(b) Those which did not show rsR' pattern. In this group, P. A. pressure ranged from 30 to 130 mm. of Hg., when intrinsicoid deflection was 0.035 sec. or more. There were 16 cases with activation time 0.04 sec. or more. All these, with the exception of only 3 cases had pressures more than 80 mm. of. Hg.

(4) Lead aVR: Even though all cases had pulmonary hypertension, R wave was absent in six cases. Of these, five had concomitant left ventricular enlargement and one was a case of constrictive pericarditis. R/Q ratio was more than one, only in 13 cases. There was no relation between R/Q ratio and the degree of pulmonary hypertension.

61. Inquiry on 'cardiac metabo!ism under hypothermia under Dr. Sitaram Kapoor at the K.G. Medical College, Lucknow.

1. The effect of various anti-fibrillatory drugs on the prevention and control of ischemic ventricular fibrillation under hypo-thermia in dogs at 25°C. was studied. Out of all the drugs studied procaine hydrochloride in doses of 10 to 15 mg. per kg. body-weight injected intra-venously 10 minutes before venous occlusion seems to be the best for the prevention of ventricular fibrillation. Once the ventricular fibrillation occurred no drug was able to control it effectively.

2. Volatile anaesthetics like ether, nitrousoxideand trilene seem to be more safer in the productien of hypothermic state than the non-volatile ones such as pentothal, dial or chloralose. Cardiac failure occurs at much lower temperatures with volatile than with non-volatile anaesthetics.

3. Asystole in a rabbit auricle at low temperatures can be maintained for about 2 hours without any detriment to the rate and amplitude of its contractions on rewarming it to 37°C.

The effect of metabolic substrates on the rate and amplitude of auricular contractions needs further investigation.

62. Autopsy study of coronary circulation in normal and abnormal hearts and study of incidence of atherosclerosis at different sites in the arterial tree under Dr. N. M. Purandare at the Seth G. S. Medical College, Bombay.

Sixty-six hearts and aortæ were studied by the injection and dissection technique, thus bringing the total number of hearts studied so far to 341. This method has proved useful in a more complete study of the coronary circulation, both in health and disease.

Of these 66 cases, 54 were males, and 12 were females. They belonged to various age groups, the oldest was 80 years of age while the youngest was 5 days old.

Fifteen hearts showed myocardial infarction. The presence of atheroma was detected in 42 cases, which are being graded, as recommended by the W.H.O. Expert Committee on Atherosclerosis. Results of a detailed analysis will be given at the conclusion of the present study which is expected to include at least 1000 cases.

63. Effect of sitosterol administration on serum cholesterol level and lipoprotein pattern under Dr. B. C. Sinha at the Medical College, Calcutta.

Control experiments on the determination of free and estrified cholesterol content of serum of 10 normal subjects aged 22 to 25 years (i.e. those who are not as yet susceptible to coronary heart disease) have been made first to find out the base line values which would enable evaluation of changes in the cholesterol content on administration of B-sitosterol.

B-sitosterol was administered to the subjects an a control diet and changes in the cholesterol content were determined. The results so far obtained on five normal subjects showed a proportionate decrease in the cholesterol content. In the case of patients suffering from cerebral thrombosis a marked decrease in the esterified cholesterol was observed on administration of B-sitosterol.

64. Enquiry on the metabolic fate of gelatin administered as plasma substitute under Dr. C. Sivaraman at the National Chemical Laboratory, Poona.

The metabolic fate of transfused gelatin and its efficacy in the treatment of shock were investigated using dogs, rabbits, rats and guinea pigs as experimental animals. It is expected that the information thus obtained will be of value in evolving a suitable method for the manufacture of 'transfusion gelatin' which is now produced on a pilot plant scale at the National Chemical Laboratory, Poona.

Physicochemical studies on transfusion gelatin showed that it has a gelling temperature of 10°C., intrinsic viscosity of 0.19, isoelectric point of 5.6 and a *number average* molecular weight of 56,000 by the light scattering method. Ultracentrifugal studies have shown that the gelatin has a *weight average* molecular weight of about 70,000.

In rats, rabbits and dogs, gelatin administered intravenously produces hæmodilution within two hours with a slight increase in the sedimentation rate and coagulation time. However, the reduction in the concentration of the formed elements becomes less marked within 24 hours. Repeated transfusions with gelatin at intervals of 24 hours showed hæmodilution but there is no evidence of any such effect when gelatin is administered at 3 day intervals.

In rats, the hæmodilution effect produced by gelatin transfusion was found to be similar to that brought about by the intravenous administration of Dextran, but the rise in the sedimentation rate and coagulation time was more pronounced with the latter than with the former.

In rabbits, rats and guinea pigs, transfusion gelatin is non-pyrogenic, non-toxic and non-antigenic and is devoid of allergic properties.

In combating experimental shock in dogs, rabbits and rats, gelatin was found to be fully effective, and in the case of rats, was slightly superior to dextran.

Studies on groups of rats maintained on a low-protein rice diet gave no indication of gelatin being anabolically utilized when the latter was given at a rate of 2 c.c. intravenously twice a week.

Excretion data from doubly depleted dogs, rats and normal rabbits after gelatin transfusion showed no species variation in the total recovery of gelatin, nearly 85 per cent of the injected gelatin being accounted for in the urine of all the three species. However, the rate of excretion of gelatin in the first 24 hours is higher in rats than in dogs and rabbits. Excretion of injected gelatin stops at the end of 48 hours in rabbits, 72 hours in dogs and 96 hours in rats

Histopathological examination of heart, spleen, liver and kidney of normal rabbits and doubly depleted rats, which were sacrificed at varying intervals after gelatin transfusion showed that intravenous gelatin does not produce any pathological changes in these organs. The chan-

ges noted in the kidneys of two out of four rabbits sacrificed one week after gelatin transfusion were obviously transient functional adaptations since such changes were not present in 5 out of 6 animals kept for a longer period of three weeks.

An appraisal of the data in conjunction with the results obtained in the laboratory on the hæmodynamic effects of transfusion gelatin indicates that this material is a promising plasma substitute.

65. Hæmatological unit under Dr. J. B. Chatterjea at the School of Tropical Medicine, Calcutta.

I. Haemoglobinopathy Syndrome

Solubility of haemoglobin.— Solubility of ferrohaemoglobulin in normals and in hæmoglobinopathic conditions was estimated following the original method of Itano. A modification of the above method as proposed at the symposium on Abnormal Hæmoglobins (1957) was also employed.

Solubility of Hb. F was highest, followed in order by Hb. E, Hb. A and Hb. S. Solubility of haemoglobins obtained from thalassaemia trait did not differ significantly from that of normals.

Quaniitation of A_2 component with a view to assess its role in the diagnosis of thalassaemia trait.—A_2 component was estimated with a Photovolt densitometer from hæmoglobin electrophoretograms obtained on thick paper (3 mm. Whatmann) with standard technique.

The mean values of A_2 component in normals, in homozygous thalassæmia and heterozygous thalassæmia were 3.2 (range 1.1 to 5.2), 8.7 (range 2.2 to 16.0) and 18.5 (range 3.8 to 22.5) respectively. In this series, A_2 component was highest in heterozygous thalassæmia.

In zone electrophoresis, A_2 and E have similar mobilities. In survey work for the detection of abnormal haemoglobins, there is thus the possibility of mistaking one for the other. In E subjects, the percentage of E component was, however, significantly higher (mean 38.4 ; range 20.5 to 65 per cent) than that of A_2 in heterozygous thalassæmia. Quantitation of A_2 component is thus useful for differentiating thalassæmia trait from Hb.E trait.

Intra-erythrocytic crystals.—Intra-erythrocytic hæmoglobin crystals as described in Hb. C disease were also observed in Hb. E thalassæmia disease and AE subjects.

Observations on splenectomy.—Splenectomy has so far been done on 28 patients, consisting of both homozygous thalassæmia and Hb. E thalassæmia with ages varying from 6 months to 24 years. The hæmatologic status of these patients was variable with hæmoglobin ranging from 1.78 to 8 gm., reticulocyte from 2 to 32, fœtal hæmoglobin from 17 to 78, plasma bilirubin from 0.4 to 3 μg., plasma hæmoglobin from 7.5 to 88 mg. and serum iron from 90 to 355 μg. per cent. During a follow up period of 6 months to 7 years, partial improvement was seen in 25 patients. Signs of improvement were : slight elevation of Hb. and R. B. C. levels, less transfusion requirements and decreased incidence of 'crisis'. In 2 patients aged 9 months and 3 years, there was no significant improvement and they died 7 months and 9 months after splenectomy, respectively. A male patient aged 24 with Hb. E-thalassæmia disease developed high fever and chest pain and died on the 3rd post-operative day.

In addition to non-specific changes, (Howell Jolly bodies and thrombocytosis), other significant effects of splenectomy were increased in the number of leptocytes, target cells and normoblasts. Splenectomy did not ultimately produce any significant change in reticulocyte count, plasma bilirubin, fœtal Hb. or plasma Hb. levels.

Splenic histology .—Weight of the spleens removed varied from 125 to 1,020 g.m. The consistency was usually firm, and in some cases fairly hard. In bigger spleens, the capsule was slightly thickened with only slight increase of trabecular tissue. Sinuses showed varying degree of dilatation. In cases where sinuses were greatly dilated, malpighian corpuscles appeared small and rather ill-defined. In most cases there was evidence of myeloid metaplasia of varying degree. The hæmosiderin content was either normal or very slightly increased. Unlike the pattern in hypersplenism, proliferation of reticulo-endothelial cells and erythrophagocytosis were not observed to any significant degree.

Hepatic histology .—The characteristic change was hæmosiderosis of varying degree. In addition there was evidence of myeloid metaplasia which, however, was usually less pronounced than that in spleen. In general, liver cells did not show any significant change. No significant activity of Kupffer's cells could be observed.

II. Haemophilia Syndrome

In a series of 181 cases of hæmorrhagic disorders investigated or followed up during the last 2 years, there were 32 patients with 'hæmophilia syndrome'. All the 32 patients showed evidence of defective thromboplastin formation. Coagulation time was prolonged in 22 cases. Prothrombin consumption was, however, poor in all cases. On a detailed investigation these 32 cases could be classified into the following groups.

Deficiency of	*No. of cases*
Antihæmophilic globulin (AHG)	21
Plasma thromboplastin component (PTC)	4
Plasma thromboplastin antecedent (PTA)	3
AHG + PTC	4

Ages of these patients varied from 2 to 55 years. Twenty one patients were aged 12 years or below. All the patients were males except one in PTA group. Twenty patients were from Bengal, 5 from Bihar, 5 from U.P.,2 from Bombay.twenty eight patients were Hindus, 1 Mohammedan, 2 Christians and 1 Parsi.Family history of bleeding was obtained only in 11 cases. Platelet count, platelet morphology, platelet function tests, levels of plasma prothrombin, factor V, factor VII and fibrinogen were essentially normal in all cases.

III. Absorption of Radioactive B_{12} Labelled with ^{60}Co

Following ingestion of vitamin B_{12} containing 0.3 microcurie of ^{60}Co, fæcal excretion of labelled vitamin B_{12} was measured over a 7-8 day

period, according to the method described by Heinle *et al.* (1952). After 3 days' interval, the experiment was repeated by adding 30 mg. of intrinsic factor concentrate to the same dose of radioactive B_{12}.

So far ten patients have been investigated. In pernicious anæmia (PA), fæcal excretion was high and the absorption poor. Addition of intrinsic factor improved the absorption. In normal subjects and in iron deficiency anæmia, more than 50 percent of the labelled B_{12} was usually absorbed. Preliminary results indicate that in NMA the absorption was definitely better than that in PA.

IV. Aplastic Anaemia

Twenty two cases were investigated and followed up. Ages of the patients varied from 7 to 57 years. The series consisted of 17 males and 5 females. Nineteen were from Bengal, 2 from Bihar and 1 from Rajasthan. History of exposure to myelotoxic agents was obtained only in one. Onset of the disease was insidious in 19 and acute in 3. All the patients showed pancytopenia of variable degree.

Blood.—Hb. varied from 1.7—7.5 g/100 c.c. R,B.C.0.5—2.3 million/cmm; P.C.V.6—24 percent; Ret.O.1—4.5; W.B.C. 1,400—5,300; Neutrophil 90—3,286 and Lymphocytes 72—3,648/ cmm.

Bone marrow.—In 20 cases, the bone marrow was markedly hypocellular; the lymphocytes and plasma cells, however, were increased. Tissue mast cells were markedly increased in 16 cases. In two cases megakaryocytes were selectively depressed.

Immunologic investigations.—Significant findings were positive Coombs' test in six, demonstrable leucocyte agglutinins in six and platelet agglutinins in three cases.

Erythrocytic fragility.—Osmotic fragility before and after incubation for 24 and 48 hours showed slightly increased resistance of the cells. Plasma haemoglobin levels estimated on sterile defibrinated blood before and after incubation showed values higher than normal in 2 cases. Mechanical fragility was also increased in 2 cases.

Alkali resistant haemoglobin.—The mean value with Singer's technique was 7.07 percent with a range varying from 3.7 to 13.6 percent.

Electrophoretogram of hæmoglobin.—The pattern was AA type in 20 and AE type in 2.

Serum iron and iron binding capacity.—Mean serum iron was 195 and the range, 94 to 360/μg percent, The unsaturated iron binding capacity was significantly reduced.

Serum vitamin B_{12}.—In 2 cases the values were less than normal being 80 and 40 $\mu\mu$g. per c.c., respectively. Both of these patients were vegetarians. Parenteral vitamin B_{12}, did not, however, improve the blood picture in either.

In others, the values were within normal range.

Serum proteins.—Total serum protein varied from 4.8 to 7.9 g. with a mean of 5.9 g. per cent. Results of fractionation of serum proteins were as shown below :—

	Mean per sent	Range per sent
Albumin		
globulins	38.4	25.4 — 56.4
	5.5	3.8 — 10.7
	10.1	5.0 — 17.1
	17.5	9.3 — 25.5
	28.6	12.7 — 38.4

V. Investigaticn on Kyasnur Forest Disease

Leucopenia of variable degree was an important feature. Leucopenia was mainly due to reduction of neutrophils, but lymphopenia and eosinopenia were also common features, Bone marrow picture showed that granulopoiesis was in general active ; evidence of maturation arrest was seen only in a few cases.

Increase of mononuclear cells—Turk's cell, lymphocytes and monocytes—was an important feature seen in all cases at some stage of the disease. Turk's cells were seen both in early and late stages; lymphocytosis was usually a late feature.

Thrombocytopenia of variable degree was another important feature. Bone marrow in every instance contained adequate number of megakaryocytes. Presence of 'inactive megakaryocytes' in the bone marrow suggested that platelet formation was not quite adequate in all cases.

'Leukoagglutinins' and 'thromboagglutinins' could be detected in peripheral blood.

There was no significant anæmia in any case. Cold agglutinins of significant titre could not be demonstrated in any case. Heterophile antibody with a titre exceeding 1 :256 was present in only one case.

Hæmostatic tests indicate that the main cause of bleeding in these cases was thrombocytopenia.

66. Enquiry on anæmias in infants and children at the Grant Medical College, Bombay.

The total number of childern investigated so far is 278. In addition 53 healthy children have been examined. A detailed report will be presented at the termination of the enquiry. In this report special stress is laid on certain important aspects of the results obtained so far.

(i) There were 12 cases of megaloblastic anæmia in this group. The incidence of this type of anæmia was maximum during the first two years of life and was associated with infection of the respiratory or gastrointestinal tract. The infants in the group were receiving breast milk. Preserved Milk or goat's milk was not being given to any of these patients. Contrary to the accepted belief that folic acid is the major deficient factor in megaloblastic anæmias during childhood, a majority of cases in the present series responded favourably to administration of vitamin B_{12}

(ii) During an intensive study of anæmias with hepatosplenomegaly, 31 families were studied in detail. Nineteen families with hereditary hemolytic anæmias were detected. There were three cases of thalassemia major, all from the surat District ; the remaining 17 cases were those of thalassemia ranging in severity from major to minor. Most of these cases were in Sindhis and people originating from Saurashtra. The necessity of carrying out field work in the area of their origin is stressed. In addition to these there was one case of hereditary spherocytosis and one of hemolytic anæmia due to Rh incompatibility.

(iii) A study of healthy children to establish average normal hæmatological standards in this part of the country was started. Initially, difficulty was encountered in persuading the parents of healthy children to permit the necessary examination.

So far 53 healthy children have been examined of the age group 7 to 9 years. Children of various age groups will be studied in the course of the remaining period of this enquiry. As the number of childern examined so far is small and restricted to one age group, no conclusions are drawn.

67. Enquiry "Experimental Studies on AcquireC Hæmolytic Anæmia" under Dr.N.N. Sen, at the Institute of Post Graduate Medical Education & Research, Calcutta.

I. Production of anti-guinea pig red-cell-antibodies in rabbits and evaluation of the nature of these antibodies developed during different periods of immunization:—

METHODS

(i) Immunization—Adult rabbits have been injected intraperitoneally with 5 c.c.of 50 per cent saline suspension of thrice washed guinea pig redcell twice a week for 4 weeks. Hæmagglutinating titre of the sera collected on each of the occasions prior to giving the injections have been determined by a number of techniques.

(ii) Techniques for the determination of hæmagglutinating titre. Initially the following methods were tried.

Saline agglutination.
Agglutination with trypsintreated red-cell.
Agglutination with papain treatde red-cell.
Agglutination with ficin treated red-cell.
Agglutination in 4 per cent P.V.P.
Agglutination in 6 per cent dextran.
Agglutinatinn in 20 per cent bovine albumin.
Agglutination in 1 per cent carboxymethylcellulose.
Agglutination using anti-rabbit globulin serum.

In the guinea pig red-cell-anti-cell system the following methods were found to be satisfactory which gave considerably higher titre than that obtained by saline agglutination method.

Agglutiuation with ficin treated red-cell.
Agglutination with 1 per cent capboxymethyl cellulose.

Subsequently the latter method using 1 per cent carboxymethylcellulose has been developed in our laboratory as a standard method for the detection of 'incomplete' antibody so that only two methods saline agglutination and agglutination in 1 percent carboxymethylcellulose have been used in the investigation of the sera collected during different periods of immunization. 0.05 c.c. of 1 percent guinea pig red-cells suspended in 0.85 percent Saline and 1 percent carboxymethylcellulose, respectively were added to 0.05 c.c. of a serial two fold dilution of sera in test tubes and incubated for 2 hrs. Agglutination in saline was observed macroscopically by gentle shaking. In case of carboxymethylcellulose 1 minute's contrifugation at 500 r.p.m. followed by gentle tapping was employed for reading agglutination.

(iii) Results of hæmagglutination titre of sera on different days during immunization,

** Days	*Titre in saline	*Titre in 1percent Carboxymethyl-cellulose.
0	0	0
3	4	16
7	32	128
10	256	1000
14	512	1000

17	512	2000
21	1000	4000
24	2000	4000
28	2000	8000
35	4000	16000

* Titre has been expressed as the average of reciprocal titre of 6 rabbits.

** Injections were given on 0, 3, 7, 10, 14, 17, 21, 24 and 28 days and sera were collected just prior to giving injuctions and 7 days after the last injection.

We have interpreted the saline agglutinating titre as representing the titre of 'complete' antibody and the carboxymethylcellulose titre as that of 'incomplete' antibody. It may be noted from the table that the serum at any stage of immunization contains a mixture of both complete and 'incomplete' antibody. 'Incomplete' antibodies have been considered by most workers to develop during early phase of immunization, though exception has been made in cases of 'incomplete' Rh antibodies which develop in the hyperimmune phase. Our results suggest that the production of 'incomplete' antibody is not the peculiarity of any phase of immunization. Whether the development of 'incomplete' antibody is a function of the nature of antigen or the animals used for immunization require to be investigated. The present worker is not aware of any previous work on the experimental production of 'incomplete' antibody against red-cell system though work on the nature and method of detection of 'incomplete' antioody found in various immunohæmolytic states has been considerable.

(iv) Results of the effect of heat on the titre of saline agglu irnating antibody and non-in-saline agglutinating antibody.Five sera frompevious experiments have been heated at 56 C. for 30 mins. 65 C. for 30 mins. and 70° C.for 5 mins.and hæmagglutinating titre in O.85percent salin and 1 pe cent carboxymethylcellulose have been determined. The result shown in the following Table.

	56°C.	30 mins.	65°C.	30 mins.	70°C.	5 mins.
	NS	Cm	NS	Cm	NS	Cm
Serum I	32	128	2	32	4	64
,, II	256	1000	4	512	4	1000
,, III	1000	4000	16	1000	4	2000
,, IV	2000	8000	16	2000	8	4000
,, V	2000	16000	16	8000	16	4000

Note :—Titre has been expressed as the reciprocal.
N.S. represents titre in O.85 percent saline
Cm represents titre in 1 percent carboxymethylcellulose.
Sera has been diluted 1 in 4 before heating at 70°C. to prevent coagulation.

From the above table, it may be observed that heat treatment of immune haemolytic serum at 65°C. for 30 min. or 70°C. for 5 min. inactivates 'complete' antibodies without appreciably affecting the non-in-saline or 'incomplete' antibody titre.

Further study on 'incomplete' antibody as to its pathologic effect is in progress.

II Pathologic effect of antiguinea pig red cell antibody.

Pathologic effects of anti-red cell antibody in the developmen of hæmolytic states have been worked out in considerable detail by many workers. In the course of experiments of the same line two interesting observations were made which will be reported here.

(a) Increasing resistance of the guinea pigs to the development of hæmolytic process with the same dose of immune hæmolytic serum and (b) development of pure erythroid hypoplasia by immune serum.

(a) Two groups of adult guinea pigs were taken for experiments each group containing 5 animals. Animals in group I were injected intraperitoneally with 2 c.c of anti-guinea pig red cell serum prepared in rabbits. This serum had a saline agglutinating titre 1 : 2000. Animals in group II were injected intraperitoneally with 2 c.c of normal rabbit serum. Animals in group I developed haemolytic anæmia with peripheral reticulocytosis and normoblastosis, decreased hæmoglobin and diminished packed cell volume of red cells and in course of 5 days 2 animals diep. The remaining 3 animals recovered in 8-10 days. Animals in group II did not show any change. The three surviving animals in group I were further injected on the 10th day with another course of 2 c.c. of immune hæmolytic serum intraperitoneally. This time the development of hæmolytic process in the three animals was very mild with slight reticulocytosis and no normoblastœmia. All the animals, however recovered within 3 days. When the animals in group II which had been previously treated with normal robbit sera were given intraperitoneal injection of 2 c.c. of immune hæmolytic sera the development of the hoemolytic process was likewise very mild with recovery within 3-5 days. This increasing resistance to the hæmolytic proeess by repeated injections of hæmolytic serum has been observed by previous workers and the mechanism has been investigated by Davidsohn(1957). Results of our investigations suggest that immunization against rabbit serum is the probable mechanism operating in the development of resistance. This investigation is still in progress.

(b) When a group of 10 adult guinea pigs were being repeated injections of increasing doses of immunohæmolytic serum intraperitoneally - 4 animals developed progressive anæmia without significant peripheral reticulocytosis. The other 6 animals, however died of hæmolytic anæmia with peripheral reticulocytosis, normoblastosis and hyperbilirubinaemia. Bone marrow examination revealed the following myeloid erythroid ratio in the two groups of animals.

		M.E. Ratio.
Group I	Animals with anæmia & peripheral reticulocytosis.	1 : 1.7
Group II	Animals with anæmia & peripheral reticulocytosis.	25 : 1

The above M.E. ratio clearly shows that anæmia in the animals of group II were the result of erythroid hyperplasia developed due to the administration of immune hæmolytic serum. Reports in the literature of immuno-aplastic anæmia of the pure red cell type have been very few. In fawer cases still the immunologic mechanism could be cleary established. Gasser (1955) feels stongly that anti-red cell antibody can certainly cause aplasia of erythropoiesis and produce 'pre red cell' anæmia. There is no experimental evidence, however, supporting this concept. Our observation is probably the first experimental evidence on the production of 'pure red cell' anæmia by immunologic means. Factors favouring the course of immune aplastic anæmia of pure red cell type, however, remain to be investigated. Further work on this problem is in progress.

76. Study on the pathogenesis of anæmia in infections under Dr. V. S. Mangalik and Dr. Sharad Kumar at the K. G. Medical College, Lucknow.

In order to obtain normal standard, 100 healthy individuals were investigated to determine plasma iron, iron binding capacity, hæmoglobin and copper content of blood. All determinations were carried out on venous blood and the individuals were not necessarily fasting. The investigated persons were mainly doctors, medical students and technicians. None of them gave a history of illness a month preceding investigation.

(a) Plasma Iron and Iron Binding Capacity in Normal Individuals:

(i) *Plasma Iron*

The mean value for plasma iron in 100 subjects was found to be 117±25 μg. per cent with individual values ranging from 67 to 200 μg. The average plasma iron was low in females and also showed a lesser per cent degree of variance. In males as many as 15 values were 150 μg. per cent or above, whereas, in females, only in 4 instances plasma iron was greater than 125 μg. per cent. In order to study the relation of plasma iron with hæmoglobin concentration in blood, the two determinations were carried out simultaneouly in 25 subjects. The amount of iron in the plasma was found to be independant of the hæmoglobin concentration under normal conditions of health.

It was considered of interest to determine the diurnal variation in plasma iron. In 9 subjects in whom this study was carried out samples of blood were withdrawn at 10 A.M. and again at 5 P. M. The third sample was obtained after 24 hours. It was observed that in the majority of the cases a fall in plasma iron took place in the evening and rose again in the next morning. An evening decrease in plasma iron was observed in 7 out of 9 individuals, the magnitude of fall varying from 3-73 μg. per cent. In the other two subjects an initially low plasma iron was seen in the morning which rose later in the evening, falling again the next day.

(ii) *Iron binding capacity* (TIBC)

The total iron binding capacity was estimated in 50 individuals. It ranged from 167-350 μg./100 c.c. with an average of 250 μg.±42. Values for TIBC below 200 μg. were observed only in five subjects and, likewise, another 5 individuals showed TIBC more than 300 μg. The average per cent saturation in the present series was 44.

(b) Plasma Iron and Iron Binding Capacity in Infections.

In all 70 patients suffering from a variety of infections have been investigated (Table I)

Patients studied.

Pulmonary tuberculosis	..	—	27.
Ulcers, abscesses and cellulitis	..	—	18.
Osteomyelitis	..	—	10.
Suppurative lymphadenitis	..	—	3.
Influenza	..	—	3.
Empyema	..	—	2.
Lung abscess	..	—	2.
Caries spine	...	—	2.
Bronchiectasis	..	—	1.
Acute epididymo-orchitis	..	—	1.
Acute suppurative otitis media	..	—	1.

Of these, 24 were patients suffering from acute and 46 from chronic infection. Distinction between chronicity and acuteness was mainly on the basis of duration of illness. Ancillary evidence, such as degree of pyrexia, the magnitude of leucocytosis, or the extent of toxæmia to differentiate between the two did not hold good in all cases. Patients with symptoms of less than 4 weeks were designated as acute, while all chronic patients had the onset of their illness a month prior to investigation. The patients were called anæmic when hæmoglobin was below 12 g. per cent.

Results.

The values for plasma iron, total iron binding capacity (TIBC) unsaturated iron binding capacity (UIBC) and hæmoglobin (Hgb.) are summarized in Table II.

Table II.

Changes in Plasma Iron, TIBC, UIBC, Per cent Saturation and Haemoglobin in Injection.

Determination. percent	Normal (50)	Infection (70)	Per cent fall from normal value.
*Plasma iron μg.	117	45	61
TIBC μg.	250	164	34
UIBC μg.	134	119	11
Saturation	45	27	40
Hæmoglobin g.	14.7	11.2	24

* Plasma iron was determined in 100 normal subjects. Figures in parenthesis indicate the number of individuals investigated.

It was observed that plasma iron and TIBC were considerably low in patients as compared with the normal subjects. An analysis of this data showed that in every case of infection, the plasma iron was below the mean normal value; it was only in one patient that the value for plasma iron approximated the standard deviation of the normal mean. It was also seen that, in general, the patients suffering from acute illness showed greater hypoferræmia than chronically ill patients. The average plasma iron per 100 c.c. in acute and chronic infections was 39 and 49 μg., representing a fall of 67 and 58 per cent, respectively from the normal mean value.

A fair degree of correlation was found between plasma iron and TIBC in both acute and chronic infections. However, the reduction in TIBC was mainly due to decrease in plasma iron and UIBC was not significantly altered.

No relationship was found to exist between the duration of infection and the extent of hypoferræmia. It was noted that in 24 patients, of illness less than a month in duration, the average plasma iron was 38 μg. per cent, whereas plasma iron in 11 patients suffering for more than a year, averaged 47 μg. per cent. Hypoferræmia, when it occured, was quick to develop and it was observed in a number of patients whose duration of illness was only of 72 hours. It persisted with the infection and after treatment, plasma iron as also UIBC and TIBC, returned to normal.

It was further seen that the reduction in the level of iron was less in afebrile cases in comparison to those who suffered from fever, the values for plasma iron in the two groups being 49 and 33 μg., respectively.

Anæmia, as an accompanying feature, was seen only in patients suffering from chronic infection. It was of moderate severity, the average hæmoglobin value in 46 patients being 10.5 g. per cent. Severity of anæmia had no relationship with the extent of hypoferræmia and the reduction in TIBC. Since anæmia developed only later in the course of the disease, many a times reduction in plasma iron existed in its absence.

69. Study of immunologic mechanisms of leucocyte abnormalities under Dr. Sharad Kumar and Dr. V. S. Mangalik at the K. G. Medical College, Lucknow.

The work under this enquiry was started in the middle of June, 1958, after the appointment of Dr. A.K. Saraya, the Research Assistant. The report, therefore, includes the work done during the period of three and a half months.

MATERIAL AND METHOD

Pure suspensions of guinea pig leucocytes were obtainted and injected into rabbits in order to prepare antileucocyte serum. The following technique was employed.

Preparation of Antileucocytic Serum :

Guinea pigs weighing 250 g. or more were injected with 15 c.c. of 5 per cent glucose buffalo meat broth intra-abdominally. Twenty four hours later the abdomen was opened. Peritoneal cavity was washed by seerile normal saline in 50 c.c. quantities successively. A milky white fluid was obtained. This consisted mainly of white blood corpuscles and only a few red blood cells. The fluid was collected in a beaker which contained few drops of 5 per cent potassium oxalate. All the fluid was transferred into a 50 c.c. centrifuge tube leaving the pieces of tissue in the beaker. The fluid was centrifuged at 1200 rpm. for 10 minutes. The supernetant was discarded and the packed sediment was resuspended in 10 c.c. of normal saline. This was centrifuged at the rate of 500 rpm. for 5 minutes and the supernetant was discarded. The procedure was repeated once again and thus the proteins were eliminated from the sample. In order to lyse the red blood cells, 5 drops of 3 per cent acetic acid was added to the cells resuspended in 10 c.c. of normal saline. The cells were washed 2-3 times with normal saline in order to remove acetic acid. Finally the compact cell sediment obtained was suspended in 5 c.c. of normal saline and injected into the ear vein of rabbits at weekly intervals for 5 to 6 weeks. All glassware used was parafinised. The suspended leucocytes had a count ranging from 0.5 to 0.6 mill/cu. mm.

The blood was obtained by cardiac puncture from immunized rabbits and the serum was separated, heated at 56°C. for one hour, and stored at - 20°C.

RESULT

The immediate effects of administration of antileucocyte serum on the peripheral blood of guineapig were studied in 7 animals.

Intracardiac injection of as little as 0.75c.c. of anti-leucocyte serum was followed by convulsions and death of the recipient animals within 5 to 10 minutes. Examination of cardiac blood post-mortem showed a profound degree of leucopenia. Subsequently, therefore, the serum was injected in amounts of 0.5 c.c. into each of 7 animals. The blood samples were examined from the ear vein at intervals of 5, 15 and 30

minutes and 1, 4, 8, 12, and 24 hours. The results are summarized in the Table.

TABLE

Immediate effect of the administration of anti-leucocytic serum on guinea pigs.

Time	Average leucocyte count 10^3 per cu. mm.	Differential Polys.*	W.B.C. Monos*
Pre-injection :	7,785	39	61
Post-injection :			
5 mins.	4,140	12	88
15 mins.	3,250	9	91
30 mins.	3,733	13	87
1 hr.	5,366	20	80
4 hrs.	4,150	26	74
8 hrs.	5,083	27	73
16 hrs.	5,033	21	79
24 hrs.	4,083	28	72

* Polys. include neutrophil and eosinophil leucocytes and Monos. Include lymphocytes and monocytes.

Five minutes after the injection of the serum, the leucocyte count had considerably decreased and large numbers of agglutinated leucocytes were seen both in the hæmocytometer chamber and stained peripheral blood smears. Maximum leucopenia was observed within 15 minutes of the injection in a majority of animals which continued for a variable period. In many of the animals, the leucocyte level did not approximate its preinjection value even at the end 24 hours. The decrease in leucocytes was mainly due to disappearance of polymorphoneuclear cells as confirmed by a differential leucocyte count. Administration of normal rabbit serum to two guinea pigs in identical doses was not followed by any significant change in the leucocyte number.

In order to study the the mechanism of leucopenia in these animals, three of the guinea pigs were sacrificed at the height of leucopenia and their tissues examined histologically. Agglutinated clumps of leucocytes were found to be present in the splenic sinusoids and some of the lung capillaries. Further studies in this direction are in progress.

70. Enquiry to find out Rh phenotypes in West Bengal and to prepare anti-Rh testing sera, under Dr. Sourin Ghosh at the Medical College, Calcutta.

During the period November and December, 1958 the following work has been done.

Rh. Phenotyping

1. 150 random samples from Bengali males have bsen tested with Anti-D, Anti-C & Anti-E sera for the determination of Rh phenotype. The following results were obtained-

CDe	......	110
CDE	......	18
cDE	......	8
cDe	......	9
Cde	......	2
cdE	......	Nil
CdE	......	Nil
cde	......	3

2. *Preparation of anti-M and anti-N sera*

Some anti-M and unti-N sera were prepared by innoculating rabbits with known M positive and N positive group O human cells, respectively. The anti-M and snti-N sera were purified by appropriate absorption treatment for other antibodies.

3. *Preparation of absorbed anti A*

Absorbed anti-A was prepared by absorbing high titre serum of group B persons with known A_2 cells. This serum is used for identification of A_1 and A_2 cells.

Preparation of anti Human Globulin Serum

Attempts were made to prepare anti human globulin serum by Prooms method. Alum precipitated group O human serum was injected to different rabbits. Unfortunately due to some epidemic, all the rabbits died before scheduled date of bleeding.

Human Immunization for Anti Rh. Sera

Some voluntary donors for immunization have been selected. The process of immunization will be started soon.

71. Liver diseases research unit under Dr. P.N. Wahi at the S.N. Medical College, Agra.

I. Adrenal Cortical Function in Human Liver Disease :

In continuation of the study started earlier, adrenal cortical function was assessed in cases of portal cirrhosis and infectious hepatitis by a battery of adrenal function tests. Since the last year's report, 12 more cases of portal cirrhosis and 16 additional cases of infectious hepatitis have been investigated. This brings the total number studied so far to 50 cases of portal cirrhosis and 47 cases of infectious hepatitis. The adrenal function tests that were performed in each of these cases were as follows :—

(i) Urinary uric acid response to ACTH.

(ii) Eosinophil response to ACTH.

(iii) Serum cholesterol response to ACTH.

(iv) Urinary 17-hydroxycorticosteroid response to ACTH

(v) Urinary 17-hydroxycorticosteroid excretion levels in 24-hour-urine specimens.

(vi) Urinary uropepsin.

(vii) Urinary 17-ketosteroids in 24-hour urine specimens.

(viii) Serum sodium.

Of these tests, two that were newly standardizep and adopte in this year's study were :–

(i) Urinary 17-hydroxycorticosteroid response to ACTH test.

(ii) Urinary uropepsin test.

The data obtained in these studies showed that while there was normal response in some of the cases of portal cirrhosis and infectious hepatitis, in others the response was sub-normal. Since the results of the above tests, except possibly serum sodium, were dependent primarily, if not wholly, on circulating levels of glucocorticoid hormones of adrenal cortex, the subnormal values obtained were suggestive of the presence of a state of hypoglucocorticoidism in a proportion of cases of liver disease.

II. Relationship of Liver Functional Impairment to Adrenal Cortical Function in Humans

In view of the fact that liver is an important site of adrenal hormone metabolism, it was considered interesting to study the relationship, if any, between functional impairment of liver and adrenal cortical hypofunction in cases of portal cirrhosis and infectious hepatitis.

For this, a battery of liver function tests were performed simultaneously with the adrenal function tests. The liver function tests that were carried out in these cases were :- (i) total serum proteins and albumin, (ii) serum cholinesterase, (iii) total serum bilirubin and direct bilirubin, (iv) thymol turbidity, (v) thymol flocculation, (vi) zinc sulphate turbidity, (vii) colloidal gold flocculation and (viii) prothrombin concentration.

The results of the liver function tests were correlated with the data obtained in the adrenal function tests in each case. This has demonst rated the existance of a close relationship between the low serum albumin levels and adrenal cortical hypofunction in portal cirrhosis. Similarly in infectious hepatitis, a relationship between the occurrence of low serum albumin and high serum bilirubin, on one hand, and adrenal cortical hypofunction, on the other, was found.

III. Study of Pattern of Amino acid Excretion in Human Liver Disease

Urinary amino acid excretion patterns were studied in 10 cases of portal cirrhosis and in 16 cases of infectious hepatitis by the paper partition chromatographic technique. These cases were in addition to those mentioned in last year's report. As reported earlier, an increased excretion of cystine, glycine, serine, alanine, glutamine, methionine and valine, is observed in a proportion of cases of portal cirrhosis and infectious hepatitis. No new amino acid spots were visible on the chromatograms.

Correlation of liver functional damage with amino acid excretion pattern showed that there was no consistent relationship between severity of liver functional impairment and occurrence of amino-aciduria in these cases.

IV. Adrenal Cortex and Amino Acid Metabolism

The relationship of adrenal cortical function to the changing status of amino acid metabolism in the various stages of evolution of carbontetrachloride-induced cirrhosis in albino rats is being studied.

A total of 300 albino rats in the weight range 80g. to 200g. are being used in these studies Injections of carbon tetrachloride, O.ICC twice weekly are given to each of them. A batch of 4-6 rats are being sacrificed every week and the following investigations done on them :—

(i) Paper partition chromatographic studies of amino acid pattern in blood and urine. Both the qualitative detection of the amino acids by the double dimensional technique and their quantitative estimation by the circular chromatographic technique are being done.

(ii) Total liver protein nitrogen.

(iii) Total serum proteins and albumin.

Simultaneously, the adrenal cortical function is being assessed by the adrenal ascorbic acid depletability technique.

Histologic studies of liver by Hematoxylin and Eosin, Reticulum, and Masson's stains and of the adrenal by H & E and Sudan stains are also being carried out.

The work is still in progress and hence detailed results are not reported at present.

V. Studies on the Clinical, Histological, biochemical and Hormonal, Aspects of Kwashiorkor.

This study which started last year, is continued and six more cases of the disease investigated. In addition to photomicrographs of the liver biopsy specimen, gross photographs of the patients were also taken.

VI. Registry of Hepatic Pathology

The Registry of Hepatic Pathology has added 100 more cases during this year, bringing the total number of cases registered to 703 till date. Each case has been indexed and given descriptive protocols.

VII. Bibliographic Index of Liver Diseases

The index is being brought up to December, 1957, with the receipt of contributions from Drs. V. R. Khanolkar, V.N. Patwardhan, P. Kutumbiah and P.N. Wahi. The contributions from Drs. B.K. Aikat and M.V. Radha Krishna Rao have not yet been received.

73. Enquiry on "blood and CSF ammonia and glutathione in liver disease with or without coma and the effect of glutamate on these levels and on the clinical condition" under Dr. Shiv Kumar at the Medical College, Amritsar.

Ammonia and glutathione levels in blood and cerebrospinal fluid were determined in 51 patients with liver disease, of whom 19 subjects were in coma. In addition, these estimations were also carried out in 6 cases of coma other than hepatic, and in 5 patients suffering from miscellaneous diseases.

The blood ammonia concentration was higher than normal in all types of cirrhosis, but it was within normal limits in cases of infective hepatitis and obstructive jaundice. It was generally raised in patients with hepatic coma. No consistent relationship existed between these levels and the clinical status of the patients. There was no correlation between the liver function tests and the blood and C. S. F. ammonia and glutathione levels, although the blood glutathione levels showed a tendency to fall and the blood ammonia levels, a tendency to rise, with an increase in the degree of liver damage.

The effects produced by intravenously administered glutamate on the clinical status of hepatic coma, were extremely variable. In no case was the coma terminated by this treatment. The therapy did initially lower the blood ammonia levels in a large number of these cases, but this effect was temporary. In some cases the blood ammonia levels rose while the therapy was still being continued.

Ammonia tolerance studies were carried out by administration of ammonium chloride in 17 patients suffering from hepatic cirrhosis and in seven Eck-fistula dogs. Similar tolerance studies, carried out in 10 normal human adults and 6 dogs, provided normal data for comparison. The blood ammonia level was assessed half an hour, one hour, and two hours after the administration of ammonium chloride, in each case. The object of this investigation was to assess the role of porto-caval collateral circulation and hepato-cellular failure on the concentration of ammonium ion in the peripheral blood. Four different types of curves were obtained. In normal controls, the peak ammonium concentration (about 4 times the average fasting value) was reached in half an hour, and came back to the fasting level within two hours. The curves were only slightly different from the normal controls, in the group of cases without established collaterals and with liver function tests yielding data lying well within the normal range. The curves were high and sustained in seven cases who had established collaterals, but in whom the liver function tests indicated either a normal or a minor derangement of function. In 4 cases, having a definite hepatocellular damage, but without established collaterals, the curves were even higher than those of the preceding group. Two patients belonging to this group were provoked into coma. The study confirmed the concept that some of the ammonium absorbed from the gut escaped into the general circulation either *via* the collaterals, thus bye-passing the liver or through the damaged liver. The need of extreme caution in carrying out the ammonium tolerance tests in patients with hepatocellular damage is also emphasised by this study, particularly in view of the fact that two such cases were provoked into coma by this test procedure.

72. Experimental study of the effects of increase in portal vain pressure with reference to the development of ascite and its relation with blood flow in the hepatic artery under Dr.R.M.L. Mahrotra at the K.G. Medical College, Lucknow.

PART I: PATHO GENESIS OF ASCITES.

Before giving a resume of the work done during the year, it may be ielpful to give a summary of the work done in the previous years under the project.

1. The work under this project was started in April 1955. During he first year of the enquiry experiments were done on normal albino rats with the object of studying the role of portal vein pressure to the occuraice of ascites. These studies revealed that partial ligation of inferioena cava above the liver is followed by massive outpouring of protein ich fluid in the peritoneal cavity. Incomplete ligation of inferior vena ava below the liver is without any effect. When the portal vein and nferior vena cava are both ligated below the liver or when partial ligation of the portal vein is done, small amount of fluid appeared in a few instanes but the effusion was never profuse. These findings suggest that for he experimental production of ascites procedures which result in venous tagnation in liver are more successful than those involving accumulation of blood in the portal or systemic veins. The study further showed that pressures in the portal vein and inferior vena cava markedly raised in a umber of animals without those having any evidence of ascites. Obvioisly, a raised portal vain pressure could not be considered as the sole actor in the occurrence of ascites.

2, During the second year of this project, the development of scites in CCL4 cirrhosis produced in albino rats was studied. The role of portal hypertension and of plasma colloid osmotic pressure in the pathoenesis of ascites in these animals was particularly investigated. The nvestigations showed that portal hypertension played no significant role n the production of ascites in CCL4 cirrhosis. Ascites was found in nimals with only slightly raised portal vein pressure. while there were nany instances where a markedly raised portal vein pressure was not ccompained by ascites. There were no important alterations in the plama protein concentrations in animals with or without ascites. In nother series of cirrhotic animals repeated plasmaphresis was done to lecrease plasma protein concentrations but this procedure seemed to iave no effect on the incidence of ascites. Similar observations were ioted in animals kept on a low protein diet. The incidence of ascites in hese experiments was, however, found to be related to the severity of iepatic fibrosis. On the basis of these observations a suggestion was nade that the occurrance of ascites in cirrhosis may be related in some way to obstruction of the outflow tract (hepatic venous system).

3. During the third year of this enquiry the effect of sodium retenion and antidiuretic hormone on the development of ascites in CCL4 cirrhosis was studied.

3.(i) Sodium retention: Series of animals were fed on a high odium diet and were also given injections of DOCA (0.15 mg. per rat) on every alternate days. Cirrhosis was produced by repeated injections

of CCL4 in these animals as in the other groups. Our findings whow that retention of sodium appears to influence the volume of fluid which accumulates in the peritoneal cavity of these animals. Snch large accumulations of fluid as found in DOCA treated animals were not observed in the untreated group. Our observations suggest that the sodium retaining hormone of the adernal cortex appears to operate at a stage when the liver is grossly damaged and when fibrosis is marked. But animals with less severe fibrosis did not develop ascites even after DOCA abministration and not all animals given DOCA and showing severe cirrhosis develop ascites. These latter findings thus, suggest that sodium retention is perhaps not primarily concerned in initiating the process of fluid transudation but appears to play an important role in increasing the volume of the transudate when the latter starts appearing in cases of cirrhosis.

3.(2) Anti-diuretic hormone :—In this group cirrhosis was produced in albino rats by repeated injections of CCL4 and antidiuretic hormone of the pituitary was administered daily during the last 5 weeks of the experiment. Our findings show that in animals treated with ADH, the incidence of ascites was not significantly higher than in the control group

4. During the current year of this enquiry the part played by lymphatic drainage from liver and aldosterone metabolism in the pathogenesis of ascites is being studied. A group of 60 albino rate have been produced. Some preliminary data on these experiments have been recorded but the final conclusions will take sometime and therefore, will be reported latter on.

PART II : NATURE OF BALLOON CELL APPEARANCE OF LIVER CELLS:—

1. The work on this enquiry was started in April 57. The balloon cell sppearance of liver cells as produced by prolonged administration of para-amino-salicylic acid was studied during this period. Albino rats were used and were fed a diet containing 5 percent of sod.PAS.Our experiments showed that balloon liver cells appear in the peripheral and the midsones of the hepatic lobules within a month of PAS administration. This change becomes diffuse and involves all the liver cells of the lobule by 4 months. If the administration of PAS is,however, continued the bolloon appearance of cells tends to disappear.

The bolloon cells show an empty cytoplasm with almost complete loss of basophilia. Histochemical studies showed that the cell is abnormally loaded with glycogen. Quantitative chemical determinations on slices of liver of such animals showed a fourfold increase of glycogen as compared to those in controls.

2. During the current year the mechanism of the glycogen infiltration in liver cells as brought about by prolonged administration of para-amino-salicylic acid has been studied. These experiments have been devised on the recommendations of the liver disease Sub-Committe meeting held at Lucknow in Dec. 1957. A study of carbohydrate matabolism during glycogen infiltration of liver in PAS treated animals has been made on following lines.

2.(1) Glucose tolerance in PAS treated animals :—A series of animals were fed on a 5 percent PAS diet for two months and then glucose tolerance was done after an intra-peritoneal injection of 12 c.c. of 3 6 percent glucose solution. Samples of blood sugar were collected at fasting, and after ½ hr. 1 hr. 2 hrs. and 5 hrs. of glucose injection. Similar procedures were done on normal control animals.

Our findings show that the utilization of parenterally administered glucose is apparantly normal in PAS treated animals.

2.(2) Changes in blood sugar during starvation in PAS treated and normal animals :—A series of animals were fed on PAS diet for 2 months, Blood was collected at full stomach, and then the animals were deprived of food. Later on, samples of blood were taken at 16 hrs., 24 hrs. and 48 hrs. The animals were killed at 48 hrs., and liver glycogen estimated. The findings show that there is depletion of liver glycogen on fasting but it is not to the same extent as in the control animals. The blood sugar during starvation in PAS treated animals, however, does not show very significant changes as compared to the control animals.

2.(3) Changes in blood sugar after injection of epinephrine. Another series of animals were fed on a PAS diet for 2 months and epinephrine was injected subcutaneously in doses of 0.08 mg. 100 mg. Blood sugar was estimated before injection, and ½, 1, 2, and 5 hrs. after injection Similar procedures were carried out in normal control animals and the findings were compared in both the groups. In the PAS treated animals there is a marked and sustained rise in blood sugar as compared to the control group. This finding appea to be of great interest and therefore, it is intended to repeat it in another group of animals.

2.(4) Enzymatic estimation :—In collaboration with the Biochemistry Deptt. of the Lucknow University, following enzymes are being studied in PAS treated and control animals, Poozphorylase, glucose 6 phosphotase and hexokinase.

74. Study of the liver in cirrhosis following ligation of the splemic artery or splenectomy under Dr. F. P. Antia at the Topiwala National Medical College, Bombay.

The study included 79 proved cases of cirrhosis of liver, of which 29 were operated.

Out of 29 operated cases, 14 were subjected to splenic artery ligation, 14 to splenectomy as they had markedly enlarged spleen, and in one case splenectomy with porto-caval shunt was performed as he had repeated hæmetemesis.

Out of 14 cases of splenic artery legation, 7 showed satisfatory clinical improvement. Of the remaining seven died of cholaemia 5 months after operation, one died of high fever and pleural effusion while two cases could not be contacted. Three died within three days of operation.

Out of 14 splenectomy cases, 13 showed clinical improvement, one expired at his residence soon after discharge due to hæmetemesis.

One patient on whom splenectomy and porto-caval shunt were done is clinically well.

The other 50 cases could not be operated upon as 35 refused operation, 4 expired due to hepatic coma, 4 expired due to hæmetemesis, 5 cases were of advanced cirrhosis with very poor state of health and nutrition. One had pulmonary tuberculosis, one had tuberculous spine. These unoperated cases are being followed as control series.

75. Investigation into some aspects of the pathogenesis of ascites with special reference to the ascites in cirrhosis under Dr. N.C. Nayak and Dr. G. S. Mohapatra at the S.C.B. Medical College, Cuttack.

Eighty eight cases of ascites included in this study were primarily divided into the following groups according to the presenting clinical features :

Cirrhosis of liver	48 cases.
Miscellaneous liver disease	5 cases.
Congestive cardiac failure	9 cases.
Nephritis types I and II	5 cases.
Malnutrition	16 cases.
Hepatosplenomegaly (unexplained)	5 cases.

In all cases detailed history of onset, duration, bleeding episodes and previous attacks of jaundice were obtained and the ascites was roughly classified into 4 grades according to the severity of effusion. Ascites of grade II & III were common (35 and 30 cases, respectively) whereas grades I and IV were found in relatively fewer cases (18 and 5 cases, respectively). Marked accumulation of fluid was found mostly in cases of cirrhosis.

A battery of liver function tests (total serum proteins, albumin and globulin by Microkjeldahl technique, thymol and $ZnSO_4$ turbidity tests, thymol flocculation, serum alkaline phosphatase, vanden-bergh and serum bilirubin) were done in all cases and punch liver biopsy was carried out as often as possible. œsophagogram and spleno-portal venography (in selected cases) were also done while the ascitic fluid from all cases was subjected to a chemical and microscopic examination.

These investigations showed that in cirrhosis of liver the most significant alteration in the hepatic function tests was a marked lowering of the serum albumin value (1.55 to 2.47 per cent) with a concurrent increase in the serum globulins so that the total serum protein values were more or less within normal range. The albumin : globulin ratio was altered to an extent of being 1 : 1 to 1 : 2. The turbidity tests showed unusually high values especially the zinch sulphate turbidity test (12 to 30 units). Serum bilirubin and alkaline phosphatase were raised only in few cases. No direct correlation seemed to exist between the serum albumin levels and turbidity values on one hand, and the degree of ascites on the other. Lower serum albumin levels and higher turbidity values were often found in cases with small effusions (grade I & II ascites), while many patients with marked ascites had only slight alterations in these values. Thus, if the increased turbidity values are taken as an indication of liver cell damage, it may be assumed that the ascites is not proportional to the hepatic cellular damage.

Liver biopsy revealed diffuse hepatic fibrosis in a majority of cases, post necrotic cirrhosis and early fibrosis with marked fatty change

in some, while in 6 cases the liver appeared normal. The latter cases showed a low serum albumin as the only biochemical abnormality (turbidity values being normal) and a rich protein content of the ascitic fluid (4.7 to 5.3 g.percent) in contrast to other patients of the same group whose ascitic fluid had low protein content (0.55 to 2.05 g. per cent). The high protein content was not associated with any other evidence of inflammation. These 6 patients responded remarkably well to hospitalization and the ascites disappered almost completely. The causeof ascites in hese patients was probably an increase in the permeabilityof the splanchnic cappilaries. The hepatic fibrosis was histologically graded into 3 grades (Grade I, II and III) and a direct relationsbip was to found exist between the grade of fibrosis and the degree of ascites. Results of radiological and clinical examination suggested the existence of portal hypertension in 4 cases only. Portal hypertension perhaps played no significant role in the pathogenesis of ascites in cirrhosis.

In the 5 cases of miscellaneous liver diseases, biopsy revealed primary carcinoma in 2, postnecrotic scarring in 2 and post necrotic cirrhosis in 1. The biochemical changes were almost indentical to the previous group except that in all 5 cases high serum alkaline phosphatase was found. In the 9 cases of cardiac failure slight change in the liver function test was found only in some cases and biopsy (done in 3 cases) showed centrizonal necrosis and occasional fatty change. The nephritis patients showed marked diminution in their total serum protein and serum albumin values. With hospitalization there was gradual disappearance of the ascites concurrent with an increase in the serum protein values. Ascites in nephritis was almost entirely due to the low serum proteins. Patients with malnutrition showed similar changes except that in 7 cases there was evidence of early fibrosis together with the fatty change, found in all 16 cases. The pathogenesis of ascites in these patients is probably same as that in nephritis. The hepatosplenomegaly cases presented uniform evidence of portal hypertension with mild cirrhotic changes in the liver. The ascites in these cases may be attributed to the portal hypertension though the role of the damaged liver as a major factor cannot be overruled.

76. Enquiry into the effects of crude liver extract used intravenously in experimental hepatic cirrhosis under Dr. L. R. Sarin at the S. M. S. Medical College, Jaipur.

As reported last year the administration of carbon tetrachloride by inhalation or subcutaneous injection or oral administration failed to produce cirrhotic changes in dogs.

The administration of carbon tetrachloride intragastrically to puppies on a fasting stomach however gave encouraging results. By administering 1 c.c. per kg. body weight on alternate days for variable length of time it was possible to produce cirrhosis in 2 out of 4 puppies used.

However, when this procedure was repeated on a fresh batch of 10 dogs (aged 7 to 9 months) all the animals died after a maximum of 22 such intragastric administrations, some died of gas gangrene of liver, while others showed an extensive hæmorrhagic necrosis with a variable degree of fibrosis not amounting to cirrhosis.

The need for revising and standardizing the technique was obvious. The earlier observations indicated that the dogs develop a tolerance to the drug, if the preliminary intragastric administrations are suitably spaced, so that subsequent alternate administrations do not bring about a fatal hepatic damage. Another factor brought out, was that the dogs showed individual variation in their response to CCl_4 which confirmed the observations of Chandler and Chopra.

Keeping these factors in view, six healthy street dogs aging from 5 to 8 months were taken and their liver function tests, done before starting CCl_4 administration (only those with normal liver function test being taken). These were kept on a standardized and properly balanced nutritious diet to rule out the contribution of nutritional deficiency in the production of cirrhosis. Carbon tetrachloride in doses of 1 c.c. per kg. body weight was administered intragastrically-first 3 doses at 3 days intervals and then 4 or more doses on alternate days. Liver function tests were repeated every 8 to 10 days after giving these courses. Spacing of further administrations was carefully guided by the degree of derangement of liver function. A gap of one week or so was given when the results of thymol turbidity and flocculation test reached a level of 4 to 5 units and ++ respectively, so as to allow the liver cell damage produced to heal by fibrosis.

Appearance of jaundice (even with normal liver function) was considered an indication for stopping the CCl_4 administrations till jaundice disappeared.

In the earlier stages liver function tests tended to return towards normal by postponing the CCl_4 administrations for 1 week or so, indicating a reversible type of hepatic damage but after maintaining the liver injury at a high level for some time by judicious administration of CCl_4 the liver function tests continued to remain abnormal.

Out of 6 dogs, 2 died accidentally in the earlier stages of the study (and were replaced by fresh dogs). One dog showed cloudy swelling and fatty degeneration with R. E. cell hyperplasia together with mild periportal fibrosis in the liver (which precede cirrhosis according to Lamsom and Bollman *et al*). The other one showed considerable degree of fibrosis, some fibrous bands uniting and encircling islands of liver cells showing atrophic changes. In one of the dogs who had an icteric episode and later recovered on postponing administration for 2 weeks, and now showing fairly persistent impairment of liver function tests liver biopsy was done in the process of which some fluid was obtained between the tongs of the needle. Though a good liver piece could not be obtained probably due to fibrosis, yet it showed bands of fibrous tissue at places focal infiltration with lymphocytes and histiocytes and focal necrosis of liver cells. Similarly on per forming the biopsy on another dog who had 21 doses some fluid was obtained between the tongs of the needle and it showed mild periportal fibrosis, cloudy swelling of midzonal cells, focal necrosis and infiltration with lymphocytes, polymophs and histiocytes and R. E. cell hyperplasia.

The dogs otherwise are fairly healthy not showing obvious ascitis or symptoms of gross hepatic damage. They are constantly observed for evidence of ill health and guarded against intercurrent infection and tick infestation.

These observations also suggest that younger dogs are more susceptible to CCl_4 than the older ones thus confirming the observations of Meyer and Pessao.

(2) An optical and electron microscopic study of the 250 specimens of experimental cirrhosis of the liver, sent from the Nutrition Research Laboratories, Coonoor.

77. Electron microscopic studies of cirrhosis of liver at the Indian Cancer Research Centre, Bombay.

This enquiry has been started in April, 1958 in collaboration with the Nutrition Research Laboratories, Coonoor and the Institute of Post-graduate Research, Calcutta. Its objects are to study isolated collagen from experimentally induced liver cirrhosis in the rat, and the fine structure of the mallory bodies, believed to be a coagulative necrosis of the cytoplasm.

During the period of review specimens of infantile cirrhosis were received for thin sectioning study. 250 specimens of experimental cirrhosis of the rat liver in formalin were received for study of the fibrous element. One hundred and twenty six of these have been processed for seven differential staining techniques for connective tissue, which are necessary towards a correct interpretation of the alterations seen in electron micro graphs of isolated collagen. A simultaneous electron microscopic observation of the collagen from these biopsies is in progress.

Proposed plan of work during 1959-60 :

(1) A thin sectioning study of the processed liver specimens sent from the Nutrition Research Laboratories, Coonoor and Department of Pathology, Institute of Post-Graduate Research, Calcutta.

COMMNUNIGABLE DILEASES.

Cholera

78. Enquiry on the evaluation of the phages acting on vibrios and application of bacteriophage typing in epidemiological investigations on cholera under Dr. M. N. Lahiri and Dr. B. Ghosh Roy at the All-India Institute of Hygiene and Public Health, Calcutta.

Object :

The enquiry has been undertaken to determine the possibility of phage typing of *V. cholerae,* and its significance in epidemiological studies. Attempt is also being made to find out the inter-relationship between agglutinable and non-agglutinable vibrios.

Synopsis of work done :

Isolation of vibrio cholerac has been made from the stools of patients, by enrichment in Wilson & Blair's fluid medium and also by plating it on Bile Salt agar medium. Ninety per cent of the strains isolated this year were of 'Ogawa' subtype. In some cases both agglutinable and non-agglutinable vibrios were isolated.

For the purpose of primary isolation of phage, nutrient broth pH 8.4 was found to be superior to pH 7.6. However, it was found that nutrient broth pH 8.4 was not satisfactory in subsequent maintenance and propagation of phage, and for such purposes nutrient broth pH 7.6 was used. The five different races of phage broadly classified last year have so far been tested on 104 strains of *V. cholerae* (44 of these are 'Inaba' subtype and the rest are all 'Ogawa' subtype). There are also a large number of strains (both agglutinable and non-agglutinable strains of *V. cholerae*) obtained this year on which the action of the phage has to be tested.

It has been observed that the phage pattern of a strain does not remain constant and this issue, however, requires further investigation.

In the course of this study a strain of phage has been obtained which has a wide range of lytic action, and when tested on 97 strains of *V. cholerae*, only 3 were found to be resistant to this phage. These refractory strains are being utilized for adaptation experiments. Study is also being carried out from this aspect to find out if this method would be helpful to type strains of *V. cholerae*.

Attempts are being made to isolate lysogenic strains of *V. cholerae* and the 2 indicator strains obtained through the courtesy of Dr. K. Bhaskaran, Central Drug Research Institute, Lucknow, are being utilized for this purpose.

79. Cholera enquiry (endotoxin) under Dr. E. K. Narayanan at the Central Research Institute, Kasauli.

This enquiry was started in 1956-57 with the object of critically examining the endotoxic theory of the pathogenesis of cholera, propounded by Dr. Burrows of Chicago. Such an examination is of very great importance from the point of view of improving the existing process of prophylactic vaccination. Opinion is slowly growing that the cellular vaccine must be modified by the addition of filtrate enzymes of *V. cholerae*.

In the first year of the enquiry, collection of fresh strains of *V. cholerae* and other organisms and their preservation were carried out. In the second year, the technique of Burrows by which he got the experimental proof of an intestinal membrane permeability-enhancing property of the endotoxin was critically examined. Investigations were also done to prepare a lysate of *V. cholerae* suitable for the tests.

In the current year, about 80 experiments have been done to test the existence of the above-mentioned property of the endotoxin, using the chloroform lysate of the organism. Since the effect of the endotoxin on intestinal membranes had been reported to vary tremendously, 47 experiments were done with the lysate of one organism and 35 with the lysate of another. It has not been possible to confirm the existence of this alleged property of the endotoxin. On the other hand, the evidence obtained showed that the chloroform lysate of *V. cholerae* reduced membrane permeability.

The growing literature on the pathogenesis of cholera has been briefly reviewed. The possible ways in which further critical examination of the endotoxic theory of the pathogenesis of cholera can be undertaken, are discussed.

80. Immuno-chemical studies on cholera with reference to Vibrio polysaccharides and proteins under Dr. Gurkirpal Singh and Dr. P. Devi at the Central Research Institute, Kasauli.

Mice protection tests with phenol-extracted "polysaccharides" from *V. Cholerae* Inaba, El Tor, rough *V. Cholerae* and non-agglutinable vibrios have shown that the first one protected mice against infection with *V. Cholerae* (four out of five mice survived), the second protected partially (two out of four mice survived) while the third and the fourth did not give any protection at all.

The phenol-extracted "polysaccharide" from *B. Aerogenes* had a high nitrogen content and contained ribose rather than fucose which others had found to be present in the polysaccharide from *B. Aerogenes.* It seems that the typical polysaccharide from *B. Aerogenes* is not obtained by the phenol-extraction method.

Other methods of preparation of polysaccharides from *V. Cholerae* were tried. Preliminary preparations of polysaccharide complexes were made from dried bacteria (a) by ether treatment of saline suspension and treatment of the aqueous phase for the polysaccharides. (b) extraction with diethyleneglycol (c) extraction with 50 per cent pyridine. Preliminary trials with (a) and (b) have given preparations containing arabinose and (c) a preparation containing ribose.

Polysaccharides have been prepared from vibrios grown in liquid medium. Three-day old cultures of vibrios grown in 1 per cent peptone water were freed from bacteria by centrifuging in the Sharpels supercentrifuge, the centrifugate acidified to pH 4.2 and the precipitate obtained further purified. These preparations still contain a lot of impurity. Preparations obtained from *V. Cholerae* Inaba and Ogawa, El Tor, rough *V. Cholerae* and non-agglutinable vibrios showed type-specifity as regards protecting mice against infection with *V. Cholerae*, Inaba. Chromatographic analyses of these preparations showed the presence of ribose sometimes mixed with arabinose.

81. Production of experimental enteritis with bacteria associated with cases of clinical cholera under Dr. S. N. De at the Medical College, Calcutta.

Eight strains of water vibrios belonging to Heiberg's Group I (4 strains), II (2 strains) and IV (2 strains) failed to produce diarrhoea. One strain of *B. typhosum* and one of *B. enteritidis* also failed to induce diarrhoea.

Rabbits immunized with ½ and 1 c.c. of emulsion (1000 x 10^6 c.c.) of *Vibrio cholerae* (living, killed at 56°C. for 15 minu'es, at 55°C. for 1 hour and at 50°C. for 15 minutes) failed to prevent experimental cholera with the same strain, though the titre of "O" and "H" agglutinin rose to 1/25 to 1/2500.

It was noticed that during the summer the rabbits were refractory to diarrhoea after introduction of *Vibrio cholerae*. Preliminary experiments suggested that diarrhoea could be induced during the summer as well, by increasing the dosage of cortisone. This remains to be confirmed.

82(a). Immuno-chemical Studies in *Vibrio cholerae* under Dr. D. L. Shrivastava at the Central Drug. Research Institute, Lucknow.

During the period under review further efforts have been made to improve the method of preparation of the polysaccharides and to study their physical, chemical and immunological properties. Samples of polysaccharide have been prepared from growths in liquid as well as on solid media.

Preparation and characterization of the polysaccharides

(a) *Broth cultures*.— In continuation of the work reported last year, a large number of samples (35) of polysaccharides were prepared from vibrios including the subtypes Ogawa and Inaba, El Tor and NAG. The procedure adopted for the extraction and purification was essentially the same as reported earlier, with slight modifications. Some of the samples exhibited precipitin titres of 2 to 3 millions.

A few control experiments were also done to see whether there was any possibility of contamination of the active polysaccharide with that originally present in the broth. It was found that the polysaccharide in the uninoculated broth was glycogen as shown by hydrolysis and chromatography. Since no glycogen was ever detected in the active polysaccharide, it was assumed that the glycogen of the broth was utilized by the growing vibro cells. This was further confirmed by respiration studies (Warburg's techniques), wherein it was seen that laboratory glycogen as well as the isolated broth glycogen gave a fairly high rate of oxygen consumption. Further work on this aspect of the problem is in progress and will be reported later.

Almost all the batches of polysaccharide (200 to 300 mg. per 12 liter batch of broth) were further studied and the results are given below:-

1. *Inorganic analysis* : Nitrogen 0.2 to 2.5 per cent.

   Phosphorus 0.2 to 0.3 per cent.

   Ash 7.2 per cent.

2. *Optical rotation*:— The ogawa polysaccharide was found to be levo-rotatory and Inaba dextro-rotatory, with varying specific rotations obtained in different batches.

3. *Ultraviolet absorption*.— Both the kinds of polysaccharides showed a maximum absorption at 260 mμ presumably due to the presence of nucleic acids.

4. *Electrophoretic mobility*.— Paper electrophoretic studies have shown the polysaccharide to be acidic in nature, because of its movement towards the anode. In spite of the different buffer systems used, a considerable trailing of the polysaccharide towords the anode was always observed and distinct spots have not yet been obtained. It was noted that the polysaccharide could be stained both by mucicarmine and toluidine blue.

5. *Rate of hydrolysis* .— The hydrolysis, as determined by the amount of reducing sugars in the hydrolysate increased up to 2 hours and remained practically constant up to 8 hours.

6. *Chromatographic analysis* .— By hydrolysis and paper chromatography presence of galactose and arabinose have been definitely established. Indications have been seen for the presence of a methyl sugar, the characterization of which is in progress.

B. *Agar cultures :*

1. Extraction of the ground cells with different buffers and salt solutions between pH 5 and 8. These in general gave poor results because when protein was separated by chloroform gel method, the polysaccharide also was found to be removed with it. This, however, is being further pursued.

2. Lysis with formamide and precipitation of the mucopolysecharide with ethyl alcohol. The precipitate was treated with either proteolytic enzymes like papain at pH 6.6 or trypsin at pH 8.5 or directly treated with alkali to hydrolyse the proteins.

The hydrolysed material was further precipitated with alcohol and the polysaccharide was isolated according to the method reported earlier. The final product was dissolved in minimum quantity of distilled water and lyophilized.

Analysis of the above samples of polysaccharide gave a high serological activity (a precipitin titre of approx. 9 millions), low nitrogen (approx 1 per cent), no inorganic phosphorus and as high as 4 per cent organic phosphorus. The suspected nucleic acid impurity is being investigated.

By hydrolysis and chromatography arabinose and galactose have been identified. Other spots are under investigation which include a suspected spot of amino sugar.

The agar diffusion technique was applied and six separate antigens could be identified in the original cell extract, out of which one was found to be heat stable.

83. (b) Cholera Enquiry under Dr. K. Bhaskaran at the Central Drug Research Institute, Lucknow.

With the evidence now available of the occurrence of genetic exchange in certain bacterial species such as conjugation (sexuality) in *B. coli*, phage-mediated transduction in Salmonella and D.N.A. induced transformation in pneumococcus and *H. influenzae*, it seemed pertinent to investigate whether any such mechanism could be demonstrated in *Vibrio Cholerae*. The essential requirement for such genetic studies was the availability of strains with differential characters which could be used as markers in genetic analysis. In *V. Cholerae* it was seen that strains differing in nutritional requirements, antigenic structure and drug resistance could be utilized for such a purpose.

When certain pairs of such genotypically different strains were grown in broth, a significant number of colonies could be isolated by selective procedures which deviated in some character or other from the parental types. The fertility of such crosses depended on the lysogenic state (phage 129) of either one or the other parent, which was suggestive of phage-mediated transduction. However, certain peculiarities of this phage rendered this improbable. Though plaques appeared in cross-matching tests of 129 lysogenic cultures and senstive strains, it was not possible to obtain hightitre preparations of this phage. Filtrates and lysates showed poor plaque counts and none at all when passed through filters of sintered glass which would retain vibrio cells. There appeared to be a close correlation between the viable vibrio count of the preparations and the plaque count on sensitive strains indicating that phage 129 may have no extra-cellular existence. However, the dependence on the lysogenic state for effective recombination suggests the role of a fertility factor, designated the P factor, played by this phage, similar to the F factor in F+ strains of *B. coli*, though the latter does not show any detectable effect of sensitive F—strains.

Crosses between P+and P – strains of *V. Cholerae* (129 lysogenic and sensitive) were regularly fertile, but the recombinant rate was reduced in P+ and P — matings. These studies were carried out in detail with strain 162 and its mutants (hypoxanthine–; hypoxanthine–; histidine–; hypoxanthine–; glutamic acid–, respectively) and strain 387 and its mutants (methionine–, aspartic acid—). Prototrophic recombinants from these crosses were isolated in minimal agar devoid of any of the growth factors required by the parent strains. It was seen that the genotype of the recombinants, as tested by certain unselected markers, were generally of one parental type. Of particular interest was the isolation of some recombinants which were Smooth derivatives of a Rough parent in the cross, and others which were unstable prototrophs continually segregating into parental auxotrophic and recombinant types.

The experimental evidence supports the possibility of 'conjugation' between *V. Cholerae*' strains.

LEPROSY

84. Leprosy research unit under Dr. N. Mukerjee at the School of Tropical Medicine, Calcutta.

I. Histological Studies

Histological study of the early reaction with refined lepromin, as compared to that with the new antigen prepared from Kedrowsky's bacillus.

Comparative study has so far been made on 25 tuberculoid and 20 lepromatous cases. After injection, biopsy specimens were taken after 24, 48 and 72 hours, and then at weekly intervals up to 4 weeks to study also the changes produced in the late reaction. The findings in general were as follows :—In the tuberculoid cases, showing positive reactions, specimens taken 24 hours after the injection showed œdema, little tissue destruction and infiltration with a few polymorphs and eosinophils, a number of lymphocytes and a few histiocytes. Specimens removed 48 hours after the injection showed focal distribution of the infiltrate with an increase in histiocytes and lymphocytes and a proportionate decrease in polymorphs, giving a picture of pre-tuberculoid histology. Those removed after 72 hours, showed aggregation of the infiltrate around the various appendages of the skin with a reduction of œdema and a further increase of histiocytes and lymphocytes at the expense of polymorphs. Small giant cells were occasionally encountered. In specimens removed at an interval of 1 week or more, a frank tuberculoid reaction could be seen. In the lepromatous cases, with negative reaction, specimens removed early showed only a slight and transient reaction which was probably of traumatic nature. Specimens removed after a week or more showed practically no reaction except some changes due to the disease process itself.

Early and late histological change observed with the two antigens were very similar in nature, in both the tuberculoid and lepromatous cases.

II. Experimental Transmission

(i) Attempt at experimental transmission of human leprosy to Syrian hamsters and black mice.

MATERIAL AND METHOD

Animals used in these experiments were all laboratory bred ; the hamsters used were 3-6 weeks old and the black mice 12-20 days old. So far 61 hamsters and 106 black mice were used. For all inoculations, a suspension of tissue free human organisms was used. Most of the inoculations were a combination of intraperitoneal and subcutaneous ones. Intracerebral, intraneural, intrascrotal and intramammary routes were utilized in a number of animals. About 1000 million bacilli were injected into each mouse and 3000 million into each hamster, the volume of inoculvm being 0.1 c. c. for the mouse and 0.3 to 0.5 c. c. for the hamster. For passage from animal to animal the number of organisms inoculated was reduced by about 1/1000. Animals

were sacrificed as a rule 6 months after inoculation, and were observed at monthly intervals up to the end of an year or more. After death or sacrifice, smears were examined from different organs which were also histologically examined. To exclude the possibility of tubercle bacillus or other saprophytes, organisms from a few animals showing infection were seeded on to the appropriate media for subsequent culture and identification.

In one instance, lepromin was prepared from the inefected liver and spleen ofi one affected black mouse following Dharmendra's chloroform extraction method, and intradermal tests were done on lepromatous and tubercu oid cases.

Of 61 hamsters, 25 died or were sacrificed within 6-14 months and 3 of these showed heavy infection, (primary inoculation-1 out of 10 ; 1st passage-1; 3rd passage-1). Of 106 black mice, 51 died or were sacrificed within 6-14 months and 17 of these showed heavy infection (primary inoculation-2 out of 24; 1st passage-4; 2nd passage-2; 3rd passage-9).

It was observed that the animals, dead or sacrificed within 6 months after inoculation showed very little progress of infection in them Animals inoculated one year or more ago showed heaviest infection-liver, spleen, omentum being enlarged and tissue cells from these being swamped with innumerable bacilli, with globus formation. Intradermal reaction with an antigen prepared from bacilli obtained from an infected mouse showed early reactions very similar to that wish Dharmendra's refined lepromin. The extensive nature of lesions observed in the tissues of black mice suggests that these animals have a greater susceptibility to human leprosy than Syrian hamsters or other experimental animals used so far. Regarding route of inoculation, intracerebral one proved unsuitable for the mice, as most of them died within 3 months. Apart from that no particular route was found to be more favourable than others.

It may be concluded tentatively that transmission of human leprosy to young hamsters and selected hybrid black mice is possible, when a limited number of tissue free bacilli from untreated active lepromatous cases is inoculated and subsequent passage carried out. In this respect the black mice were found to be more suitable than the hamsters. (*ii*) Transmission of human leprosy into white rats by intraneural inoculation.

Three groups of white rats, 5 in each group, were inoculated with 0.1 c.c. of the following : (1) normal saline (2) a suspention of human leproma in saline and(3) an albuminized suspension of the same. Inoculations were given into the sciatic nerve by open dissection method. The two suspensions used were very rich in bacilli.

One rat of the third group, developed a small subcutaneous fluctuating swelling over the right side of the chest, 3½ months after inoculation, and appeared to be ill. It was, therefore, sacrificed; spleen, liver and axillary glands were found to be enlarged. A caseous abscess was found over the chest wall. Smear from liver, spleen, lymph glands and the caseous material revealed enormous number of acid fast bacilli. The

caseous material was also inoculated into Lowenstein-Jensen, Petroff and glycerine agar media. No growth occurred. It has also been inoculated into guinea-pigs which are still living.

It is too early to say that the organism encountered in the above white rat is Myco-leprae hominis.

III. Therapeutic Studies

(i) Evaluation of S. U. 1906 (Ciba) (D.P.T., 1-(P-butoxyphenyl) 3-(P-dimethylaminophenyl)-2- thiourea) in the treatment of leprosy, and comparison of its effect with that of DDS. A comparative study of D.P.T. in 13 cases of leprosy (lepromatous 6 and neural 7) and DDS in 12 cases (lepromatous 6 and neural 6) had been undertaken. D.D.T. has been used orally in dose of 125-1000 mg. per day and DDS. in the usual doses o 10-100 mg. per day. The treatment has so far been given for 9 months. It has been observed that clinical response with D.P.T. is as good as that with DDS although bacteriological improvement has been better with DDS. The drug is not found to be toxic in the doses used. These findings are in conformity with some of those published earlier by Ross Innes (1957) and Davey (1958).

(ii) *Peroxidised hydnocarpus oil.*—Three cases (L-2-1) have been treated with local application of the above in doses of 5 c.c. daily. Treatment has been continued so far for 6 months. No clinical or bacteriological improvement has been observed in any case during this period. The claim of Prof. Baranger that the hydnocarpus oil when peroxidised is better absorbed through the skin and produces both a local and a general beneficial effect could not, therefore, be substantiated in this limited trial.

85. Leprosy Enquiry under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay.

I. *Studies on the Lepromin Reaction.*—A number of different antigens were used as alternatives to lepromiin in an attempt to determine their suitability for replacing the latter. This was in view of the difficulty encountered in obtaining sufficient number of lepromatous nodules for preparation of standard lepromin

Among the antigens tested in a total of 235 patients were (a) an antigen prepared from defatted Kedrowsky bacilli (b) an antigen prepared from cultures of an acid-fast organism obtained from leprosy patients in Bombay, and (c) an antigen prepared from freeze-dried cultures of Leishmania tropica. Clinical observations were supplemented by a histological examination in a large majority of cases, "A.L.H. Lepromin" was used as control for these antigens.

The results so far, indicate that only the antigen prepared from Leishmania culture behaves to some oxtent like the standard lepromin.

II. Complementary to the above studies, was a preliminary investigation on the tissue reaction to chemically inertparticulate matter (glass wool powder), administered intracutaneously. It is interesting to note that, while distinct cutaneous nodules were evoked in 67 out of 99 tuberculoid subjects, there was no detectable clinical reaction in 58 out of 62 lepromatous patients.

III. Clinical and histological observations on 101 contacts of leprosy patients have been carried out as before. 10 cases have developed lesions of tuberculoid leprosy.

IV. *Therapeutic studies.*—Therapeutic trials were undertaken with two indigenous products prepared by Himalayan Drug Company, *viz.*

(i) Rumalya for bone and joint pain in 15 patients, and

(ii) Nardyl for nerve pain in 8 patients.

86. Leprosy Enquiry under Dr. Paul W. Broad, at the Christian Medical College, Vellore.

OBJECT :

(i) To investigate the relationship between the fundamental pathology of deformity in leprosy and the secondary exciting factors.

(ii) To explore methods of prevention of deformity by eliminating the immediate precipitating factors, which seem mostly to be physical and mechanical in nature.

(iii) To devise methods of repair and reconstruction of deformed faces and limbs.

Conclusions already drawn .— Reports have already been presented of the predictability of much of the deformity in leprosy. The relative vulnerability of different nerves and muscles has been studied and certain factors, such as variations in temperature near the surface of the body, have been shown to be important in the production of deformity.

A programme of physiotherapy has been worked out and published, which may be used in limiting secondary deformity and in the correction of existing deformity. Series of splints have been designed for the correction of deformity which are being manufactured.

A large series of operations have been devised and some existing ones modified, so that the majority of leprosy deformities which are not preventable can be appropriately corrected. These operations are still being modified to produce better results and new operations are being added.

During the past year, detailed follow-up studies have been published on patients operated for claw hand by the Bunnell sublimis transfer method and 150 cases operated by the new free grafting technique for correction of claw hand, have been followed up. This operation has already been summarized in a previous report. The follow-up studies revealed that the new operation is by far the most successful that has ever been used for this condition. The method of free grafting which has been devised under this enquiry, has been reported at the annual meeting of the British Orthopædic Association, together with a film show to demonstrate the detailed technique. The method was well received by the British Orthopædic Association and the film was accepted for the International Film Library sponsored by CIBA Foundation. It is believed that this tendon grafting procedure will be very useful in many conditions other than leprosy, e.g., polio, nerve injury, etc.

The thumb reconstruction procedure that has been used for some years, has now been modified at the suggestion of Barr of Boston in order to prevent the secondary deformity which has become apparent in some of the earlier cases. This modification has proved successful in preventing flexion deformity of the terminal joint, and instability of the metacarpophalangeal joint.

Surgery of the face.— One of the gross deformities of leprosy is the collapse of the nose resulting in the very obvious and distressing mark of the disease. A number of different operations have been tried for these cases under this enquiry, but with no regular success. Advice has been sought from Sir Harold Gillies, Jack Penn of Johannesburgh, William White of Pittsburgh, and finally from Dr. Antia of Poona, who has successfully applied the principles originally suggested by Sir Harold Gillies. Dr. Antia's results have been good. The chief difficulty however, is that the co-operation of an expert prosthetic mechanic is required. Dr. Antia's basic methods have been adopted with an attempt to avoid the need for complex prostheses, using earlier bone grafting instead. The results so far have been encouraging. Within the next year or two, it may be possible to ensure complete correction for any nose damaged by leprosy.

Surgery of the feet.— Trophic Ulceration : This aspect of the work has been transferred to the Research Enquiry at Karigiri.

Rubber shoes have been produced experimentally by means of spraying pre-vulcanised latex on to a close-fitting sock, and thus partially reproducing the conditions that prevail inside a plaster-cast. Preliminary trials with these shoes have not been very successful because of friction effects on the dorsum of the feet.

The use of microcellular rubber (produced in Japan), is also being investigated and it seems likely to be valuable in the prevention of localised pressure of high-risk areas on the sole of the foot.

87. Leprosy Enquiry under Dr. N. Figueredo at the Acworth Leprosy Home, Bombay.

I. Examination of New Contacts.

(a) Examination of 257 family members of neural patients revealed that 27.2 per cent were uninfected. 52.9 per cent were infected without lesions, 8.1 per cent had the earliest lesions and 11.8 per cent had already contracted leprosy.

(b) Examination of 179 family members of lepromatous patients revealed that 20.7 per cent were uninfected, 50.2 per cent were infected without lesions, 5.0 per cent had the earliest lesions and 24.0 per cent had already contracted leprosy.

II. Re-Examination of Contacts of Previous Years.

(a) Development of lesions.

Re-examination of 49 contacts over a period of 8 months to 8 years 3 months revealed the following :

(1) Nine out of 29 of the uninfected got infected (without lesions).

(2) Five out of 29 of the uninfected and 1 out of 20 of the infected (without lesions) contacts developed primary lesions.

(3) One out of 29 of the uninfected 3 out of 20 of the infected with out lesions and 3 out of 11 of the contacts with primary lesions developed macules with sensory impairment.

(4) Fourteen out of 29 of the uninfected contacts remained uninfected.

(5) Three out of 20 of the infected (without lesions) contacts remained so.

(b) Regression of lesions.

Re-examination of 45 contacts over a period of 8 months to 8 years 3 months revealed the following :

(1) Seven out of 20 of infected (without lesions) contacts became negative for bacilli (Period : Min. 1 yr.; Max. 5 yrs. 2 mths.).

(2) The primary lesion vanished in 3 of the 5 contacts with such lesions (Min. 5 mths.; Max. 1 yr. 11 mths.) but they continued to be positive thereafter for periods of 3 mths. to 1 yr. 11 mths.

III. Prophylaxis With D.D.S.

Dose Schedule : Contacts	under 5 yrs. 5	mg.	twice a week
,,	from 5 to 10 yrs.....10	mg.	-do-
,,	,, 11 to 15 yrs. ..25	mg.	-do-
,,	over 15 yrs. 50	mg.	-do-

(A) *Detailed study with Laboratory Investigations :*

One hundred thirty seven contacts on regular prophylaxis have been observed during a period of 1 to 4 years. The conditions noted from time to time in these contacts are presented below, together with a comparable group of untreated contacts.

(I) *Negative Contacts* (uninfected)

Among the negative contacts observed during a period of 1 to 4 years:

(i) Out of 57 on prophylaxis, none developed lesions. 6 become infected, of which 2 were bacteriologically negative but were considered infected because of the development of lepromin positivity, and 51 remained uninfected.

(ii) Out of 58 on control, one developed macules with sensory impairment, 3 developed primary lesions, 17 became infected and 27 remained uninfected.

(II) *Positive Contacts* (infected without lesions)

Among the positive contacts observed during a period of 1 to 4 years :

(i) Out of 53 on prophylaxis, none developed lesions, 33 remained infected and 20 became negative.

(ii) Out of 53 on control, one developed macules with sensory impairment, 2 developed primary lesions, 40 remained infected and 10 became negative.

(III) *Contacts with Primary Lesions* (macules without sensory impairment)

Among the contacts with primary lesions observed during a period of 1 to 4 years:

(i) Out of 28 on prophylaxis, 3 developed macules with sensory impairment, 14 remained static, 4 remained infected but in these, the primary lesions vanished and 7 became negative for bacilli and the primary lesions also vanished or faded.

(ii) Out of 32 on control, 4 developed macules with sensory impairment, 14 remained static, 9 remained infected but in these the primary lesions vanished, 5 became negative for bacilli and the primary lesion also vanished or faded.

(B) *Field Study* (without laboratory investigations)

One hundred two lepromatous and 72 neural household are at present under observation. Of the lepromatous contacts, 201 are on prophylactic treatment and 203 are kept as controls. Of the neural contacts, 144 are on prophylactic treatment and 129 are kept as controls. The majority of the above contacts have been under observation for a period of 2 to 2½ yrs.

To date none of the contacts from the prophylactic group have developed definite lesions while from the control group 4 (2 from lepromatous households and 2 from neural households) have developed definite lesions of the Nt type.

IV. The Influence of Tuberculous Infection on Lepromin Reactivity.

(A) A comparative study of the lepromin and tuberculin reactions in 473 contacts of leprosy patients has shown a higher percentage (10 to 17 per cent higher) of lepromin positive reactors among the tuberculin positive contacts as compared to the percentage of lepromin positive reactors among the tuberculin negative contacts. However, a considerable percentage (varying from 50 per cent in children from 0 to 6 yrs. of age to 15 per cent in adults of 25 yrs. and above) of tuberculin positive contacts remained negative to lepromin.

(B) A similar study in 108 tuberculosis patients (all tuberculin positive) revealed that 60 per cent of them were positive to lepromin. In 63 of these patients in whom classification of tuberculous lesions was carried out, it was observed that (i) of the 29 patients with minimal lesions, 15 were positive to lepromin (ii) of the 24 patients with moderately advanced lesions 17 were positive to lepromin and (iii) of the 10 patients with far advanced lesions 9 were positive to lepromin.

These results reveal that while mild infection without disease with the tubercle bacillus (or the lepra bacillus) is sufficient to induce reactivity to the homologous antigen in the majority of individuals, heavy infection (with tuberculosis) is necessary to induce an equivalent index of reactivity to the heterelogous antigen (i. e. lepromin) which fact thus explains the occurrence of individuals tuberculin positive and lepromin negative and at the same time demonstrates that leprous infection is the most potent factor in the induction of lepromin positivity.

V. Tuberculin Reactions in Leprosy Patients

(1) Out of 119 neural patients tested with 5 T. U., 93 were positive whereas out of 69 lepromatous patients similarly tested 48 were positive.

(2) Out of 40 neural patients positive to 5 T. U. who were re-tested with 0.1 T. U., 7 were positive, whereas out of 8 similar lepromatous patients similarly re-tested 2 were positive.

(3) Out of 21 neural patients positive to 5 T. U. who were re-tested with 0.5 T. U., 17 were positive, whereas out of 16 similar lepromatous patients similarly re-tested 9 were positive.

(4) Out of 23 neural patients negative to S 5 T U who were re-tested with 250 T. U., 17 were positive, whereas out of 6 similar lepromatous patients similarly re-tested, 5 were positive.

VI. Follow up of the Lepromin Reaction in Leprosy Patients under Treatment with D.D.S.

A. *In Lepromatous Patients*

A re-examination of 68 lepromatous patients, originally strongly bacteriologically positive and lepromin negative, after 4to7 years of sulfone treatment gave the following results :—

(i) In 22 patients negative for bacilli by the routine as well as the Chloroform Extraction Method, the test was—ve in 2, ± in 5, +in 13 and +± in 2.

(ii) In 25 patients negative for bacilli by the routine method but positive for 1 to 10 bacilli/200 fields by the Chloroform Extraction Method, the lepromin test was — ve in 6, ± in 12 and +in 7.

(iii) In 10 patients positive for 1 to 3 bacilli/200 fields by the routine method and positive for 11 to 30 bacilli/200 fields by the Chloroform Extraction Method, the lepromin test was — ve in 4, ± in 5 and + in 1.

(iv) In 11 patients positive for 6 to 10 bacilli/200 fields by the Routine Method and for 40 to 100 bacilli/200 fields by the Chloroform Extraction Method, the lepromin test was — ve in 9 and ± in 2.

The development of lepromin positivity in only those patients who show an almost complete disappearance of bacilli suggests that this development, far from being an indication of the development of resistance as hitherto supposed is in fact the effect of bacillary regression to a certain minimal concentration as that obtaining in infected contacts, tuberculoid and certain other forms of non-lepromatous leprosy. in other words that the lepromin negativity of the lepromatous patient is the consequence of the *in vivo* "desensitization" of an originally lepromin positive stage by the excessive number of bacilli.

B. *In Neural Patients*

(i) In 18 patients with early neural lesions (Ns or Nt) whose lesions regressed and who became negative for bacilli after 2to6 yrs.of treatment, the lepromin test showed the following :—

(a) Sixteen showed a reduction in the degree of the reaction varyin gfrom 1 mm. to 7 mm. with an average of 2.4 mm.

(b) In 2, the reaction increased by 1 mm.

(ii) In 6 patients with early neural lesions (Ns or Nt) whose lesions remained static or regressed, but who remained positive for bacilli after 2 to 6 yrs. of treatment (irregular) with D.D.S., the lepromin test showed the following :—

(a) In 1, the reaction decreased by 1 mm.

(b) In 4, there was no change in the degree of the reaction.

(c) In 1, the reaction increased by 1 mm.

This significant reduction in lepromin positivity in only those individuals showing bacteriological regression suggests that the continued maintenance of lepromin positivity requires a continual immunological inducement in the form of lepra bacilli.

88. Metabolic studies of human leprosy under Dr. (Miss) B. M. Braganca at the Indian Cancer Research Centre, Bombay.

During the year under review investigations carried out on the above enquiry form a continuation of the preliminary findings reported last year. Studies have been conducted on the metabolic properties of Mycobacterium leprae with a view to ascertain particularly the enzyme pattern by which these organisms are able to metabolize various substrates to obtain the energy for vital processes. Biochemical investigations on the bacilli require fairly good quantities of material in a pure form. During the first stage of the work it was, therefore, necessary to concentrate on the development of methods to separate the organisms in a pure form and in sufficient quantity, from lepromatous nodules. This was carried out successfully by differential centrifugation procedure using aqueous solvents which are known not to denature proteins. It was found that the separated organisms showed consistent respiration indicating that the enzymes involved were metabolically active. The oxidation was determined by the conventional Warburg manometric technique. Experiments with the addition of a heated extract prepared from nodular tissue showed increased oxygen utilization over the endogenous respiration. Since it is well known that the biological systems concerned with over-all oxygen uptake are very complex and involve several enzyme systems it was of interest to investigate the occurrence and activity of individual enzymes concerned in oxidation processes. The demonstration of the enzyme *cytochrome oxidase* was reported last year.

During the current year it has been possible to identify, for the first time, the presence in M. leprae of a few individual enzymes which are involved in the respiratory pathway. Since it was found that the lipoidal capsule present in the Mycobacteria interfered with the penetration of specific added metabolites, the separated organisms were further subjected to a process of crushing. By this procedure it has been possible to demonstrate utilization of the added materials. *Succinic dehydrogenase and lactic dehydrogenase* were studied by measuring the increase in oxygen uptake using succinate and lactate respectively as substrates. The results obtained with the above substrates suggest that these enzymes are present in the human leprosy bacilli. Further, cytochrome C appears to be a carrier in the oxidation of succinate, and cyanide is found to inhibit the system. It is, however, observed that the inhibition produced by cyanide decreases with time. The reason for this is not clear, but it suggests that the oxidation of succinate may have more than one enzymic pathway. *Cytochrome reductase* activity of the bacilli was studied using a spectrophotometric method. The results obtained indicate the presence, in these organisms, of the enzymes which reduce oxidized cytochrome C. Preliminary experiments, in which reduction of diphosphopyridine nucleotide (DPN) was measured spectrophotometrically, have shown that lactate oxidation is mediated by DPN. Moreover it, has been found that one of the products of lactate oxidation is pyruvate. The pyruvate was determined by the carbonyl test using 2 : 4 dinitrophenyl hydrazine. In the respiration experiments with lactate, it has been observed that the greater the oxygen uptake the smaller is the accumulation of pyruvate. These results suggest that the enzymes concerned with pyruvate oxidation are present in the human leprosy bacilli. It may be mentioned that studies of the various factors involved in the oxidation of lactate, and in pyruvate oxidation, are presently in progress.

In view of the hopeful results already obtained on the enzymology of M. leprae it is proposed to continue the investigations on the various components concerned with the respiratory metabolism of these organisms. When the presence of several of these enzymes has been established, and some of the intermediate products formed investigated, it will be possible to join the various biological links and have an understanding of the peculiarities, if any of the metabolic pathways of the human leprosy bacilli.

89. Enquiry to study the effect of denervation on the normal physiological responses of the blood vessels of foot, in leprosy with a view to determining the state of nutrition of the tissues of the foot in relation to their liability to ulceration under Dr. E. P. Fritschi at the Schieffelin Leprosy Research Sanatorium, P. O. Karigiri, North Arcot, Distt. Madras State.

Section I.

Study of reflex vaso-dialation was made in a group of 9 patients and 6 healthy controls. The patients were all cases who had chronic trophic ulceration and whose ulcers had healed.

Investigation shows (1) that the initial temperature of the feet of the patient group was higher than that of the control group when both had been at rest in cool for at least one hour. (2) The rise in temperature on heating the upper extremities was negligible in the patient group being less than 1 degree while the same was between 4 degrees and 6 degrees in the control group.

It is concluded that vaso-motor mechanism is defective in feet which are ulcer prone and in such feet the response of the blood vessels to alterations of environments is to a large extent abolished.

Section II.

The experiments in different qualities of material to be used in making shoes for patients with healed ulcers has continued. Materials have been leather with sponge rubber and pads, moulded alkathene and very recently a special micro-cellular rubber. Careful notes are being maintained on the quality of shoe given in relation to the recurrence of ulceration.

MALARIA

90. Enquiry on the control of filariasis (W. Malayi) in Shertallai, Kerala State, under the Director, *Matorial Institute* of India, Delhi.

Follow-up studies were carried out in the area where mass therapy 30 with diethylcarbamazine was done during last year. In addition some persons who were put on two differeut schedules of treatment some 24 to 30 months ago were followed up. Periodicity studies of *W. malayi* were also undertaken.

RESULTS

Follow-up studies in the pilot mass therapy and comparison areas :

The mass therapy area (Kadakkarapally) and the comparison area (Thannirmukham) were re-surveyed in May-June 1958. Monthly clinical enquiries were done in both the areas to obtain data regarding the prevalence of filarial disease *viz*., filarial fever, lymphangitis and lymphadenitis, after adopting control measures. Entomological investigations like mosquito density, infection and infestation rates were also done in both the areas.

In Kadakkarapally 4032 persons were surveyed in 1958 as compared to 4622 in 1957.

The overall infection and disease rates in 1957 were 18.0 and 11.5 per cent, respectively, while in 1958 (after control) the rates were 16.5 and 11.5. The average infestation rate which was 20.2 before control, came down to 15.2.

In persons who had taken a complete course of therapy for 5 days the infection rate came down from 17.2 to 5.2, the average infestation from 19.2 to 8.0, while the disease rate remained more or less stationary. Reduction in infection rates and average infestation was also noted in persons taking the drug for varying days.

An analysis of persons who were initially negative and became positive in the 1958 survey showed that in the persons who took 5 days course it was 1.7 per cent, while amongst those who took the treatment irregularly, the same was 2.3 per cent. In the untreated group the percentage of new cases was 4.6 This would indicate either fresh infection or the lack of effect of the drug on the developing forms of infection acquired prior to therapy or both.

Analysis of the frequency distribution of microfilariæ in persons who took full course as also those who took therapy for varying number of days has shown considerable reduction in the possible quantum of transmission. Whereas 100 per cent of the persons were positive to infection prior to therapy 73.1 per cent of them remained negative even at the end of one year after treatment.

In the comparison area Thannirmukham, 1581 persons were surveyed in 1957 as compared to 1742 in 1958.

The overall infection, disease and endemicity rates were 12.2, 8.0 and 20.7 per cent, respectively, in 1957 and 11.6, 6.4 and 17.8 per cent, respectively, in 1958.

Monthly clinical enquiries revealed that percentage of new cases was higher in the group under therapy than either in those without therapy or in the central area. The attack rate per sufferer was the highest in control area followed by that observed in people who underwent mass therapy. A surprising feature, however, was that the attack rate was the lowest in those who did not take therapy in the mass therapy area. The possible role of therapy as a contributary cause in this connection has to be considered.

Entomological Studies :

Collection and dissection of mosquitoes were carried out in the experimental and control areas. It was observed that there was no difference in the proportion of mosquitoes with infective larvæ in the two areas.

Follow-up studies of pilot dose-schedules :—

(a) Of 24 persons initially positive and put on a dose-schedule of 4 mg/kg. daily of diethyl carbomazine for 5 days, 10 were noted to be still positive 30 months after therapy. In no case was the original count attained, the average infestation having fallen from 19.8 to 2.2 mf. per 20 cm/.

(b) Of 8 persons initially positive and put on a dose-schedule of 6mg /kg. daily for 3 days only one was still positive when examined 2 years after therapy. The average infestation had fallen from 20.4 to 2.0 mf per 20 cmm.

Periodicity Studies :

In five persons periodicity of *mf. malayi* was studied. In one case samples were obtained two hourly by day and hourly by night. In others four hourly by day and two hourly by night, samples were obtained. Two cases showed microfilariæ in all samples taken during 24 hours. Generally two peaks, one between 10 p.m. and 12 midnight and the other between 4 and 6 a.m. were noted.

91. Studies on the general behaviour of vector species of Anophelines at the Malaria Institute of India, Delhi.

1. *Techniques for measuring the susceptibility of insects to insecticides.*

Satisfactory techniques for determining the insecticide susceptibility of *C. fatigans*, *M. annulifera* and bed bugs have been worked out. The susceptibility of malaria vectors, other anophelines, culicines and mansonioides has been determined in various parts of India and the mean tolerance distribution worked out. (number of MLC's of different species determined were 84 for DDT, 28 for Gamma BHC and 43 for dieldrin). The laboratory studies on the development of resistance to DDT in *A. stephensi* has confirmed the previous findings that selection pressure applied at both larval and adult stages hastens the development of resistance. A resistant strain of *Culex fatigans* has been selected which thrives in a cage treated with D. D. T. at 200 mg. per sq. ft.

2. *Investigations on the genetic basis of resistance.*

The investigations on the genetic basis of resistance have shown that a cross between *Culex molestus* of Germany and *Culex fatigans* of India and Malaya is not possible. The inter specific cross between Indian and Malayan strains of *Culex fatigans* has revealed that maternal factor is involved in determining the crossability. DDT resistance in *C. fatigans* is found to be due to a single recessive gene.

A mutant *Culex fatigans* with short wings has been obtained as a result of radiation with x-rays. It would debate appear that the source of blood meal has a definite connection with the development of eggs.

3. *Investigations on the biochemical basis of resistance.*

The resistance in *A. subpictus* is due to dehydrochlorination of DDT. So far, such studies have been carried out on flies and culicine mosquitoes. There seems to be no reference in literature on anopheline mosquitoes except for a personal communication by Kearn on *A. sundaicus* as recorded by Brown, 1958. (WHO/Insecticide/76).

92. Pilot studies on the control of drancontiasis (Guinea worm) in India under the Director, Malaria Institute of India, Delhi.

The object of the investigation is to carry out pilot studies on the possible use of Draconticidal drugs and other lethal agents against the cyclops in the control of Dracontiasis.

The Assistant Research Officer who was appointed, resigned due to illness. Arrangements for recruitment of a suitable substitute and a Research Assistant have been made.

During the year literature on Dracontiasis and its control has been collected. Arrangements have been made for procuring samples of insecticidal formulations incorporating bentonite and molluscicidal agents, for laboratory and field trials.

93. Tuberculosis Survey under Dr. P. V. Benjamin, Tuberculosis Adviser to the Government of India, Directorate-General of Health Services, New Delhi.

This survey was undertaken in six zones around centres which had mobile x-ray units and facilities for bacteriological examination. Four centres, namely, Union Mission Tuberculosis Sanatorium, Madanapalle, and the Tuberculosis Centres at New Delhi, Patna and Trivandrum had both these facilities. At two other centres, namely, All-India Institute of Hygiene & Public Health, Calcutta, and the Tuberculosis Centre, Hyderabad, facilities for bacteriological examinations had to be organised.

The object was to secure information about the prevalence of tuberculosis in a cross-section of the population, representing urban and rural communities, as well as different age and sex groups. The information thus gained, it was thought, could be used to infer the magnitude of the problem for the entire country.

The survey was done on a sampling basis which covered cities, towns and villages accessible to the mobile x-ray unit. The examination consisted of taking x-ray film (miniature) followed by bacteriological examination in those showing suspicious x-ray shadows. The x-ray films were read independently by two local medical officers in each zone and a sample of those classified as abnormal by one or both of them was read by a group of six senior officers central. The final decision regarding tuberculosis prevalence was made on the basis of the radiological findings by the senior workers and the results of the bacteriological examination.

The field work was completed as originally planned in five of the six zones but in one zone, *viz.*, Calcutta, the whole sample could not be completed as orginally planned. In this zone, however, the survey covered the city of Calcutta, three towns and three villages. About 90 per cent of the selected population was examined.

The salient findings of the survey may be summarized as follows :—

(1) Prevalence rate for "active" and "probably active" tuberculosis varied from 13 to 25 per 1000 population in cities, towns and villages in the different zones.

(2) The rate of bacteriologically positive cases in these areas varied from 2 to 8 per 1000 population.

(3) Prevalence rates in the cities, towns and villages were generally of the same order.

(4) Prevalence rates were lower in females than in males, specially in age groups above 35 years.

(5) In general, the prevalence rate showed a gradual increase with age.

(6) In the cities, the higher prevalence among persons living in kutcha houses as compared to those in pucca houses indicated the possible effect of economic and environmental conditions.

(7) A large majority of the "active" and "probably active" cases had moderately advanced disease.

(8) Definite cavitation was observed in 4 to 33 per cent of the "active" and "probably active" cases, this percentage being generally smaller in the cities.

The survey showed that the differences, if any, in the morbidity rates between cities, towns and villages were much smaller than expected.

This finding emphasises the need for having a detailed study of the spread of tuberculosis with special reference to the ecology of populations living in cities, towns and villages.

94. Scheme of Tuberculin retesting of persons vaccinated in the mass BCG campaign in India under Dr. P. V. Benjamin, Tuberculosis Adviser to the Govt. of India, Directorate General of Health Services, New Delhi.

During the period under report about 9,000 school children in ten States were re-tested. This showed that satisfactory degree of allergy was being imparted during mass BCG Vaccination. The average induration among those retested was 13 5 mm., and in 30 out of 91 groups, the mean induration was 14.00 mm or more.

Children of age group 10 to 18 years showed higher induration than the of the younger age group (5 to 9 years). Similarly girls generally showed higher induration than the boys. These differences, however, were not statistically significant.

Presence of keloids at the site of vaccination was noted in some regions and this was as high as 42 per cent in one region.

Re-testing was also done in a group of vaccinated persons among the families of tea estate workers in West Bengal. The interval between vaccination and re-testing varied from 4 months to 8 years. The over-all mean induration for this group was 13.6 mm. Though some information on the allergy induced in general population has been obtained by this study, need for further studies on larger numbers is felt.

Two special studies, one on the status of allergy in the period between vaccination and re-testing and the other, on the boosting effect of repeated tuberculin tests are progressing as scheduled.

A pilot study in three villages has shown that it would be possible to extend the re-testing programme to the vaccinated groups in general population also and it is considered that such studies will make the assessment more reliable.

95. Tuberculosis Chemotherapy Project in Madras under Dr. P. V. Benjamin, Tuberculosis Adviser to the Government of India, Directorate General of Health Services, New Delhi in association with the WHO BMRC.

The Tuberculosis Chemotherapy Project, Madras, is being conducted under the auspices of the Indian Council of Medical Research in collaboration with the World Health Organization and the British Medical Research Council. An agreement governing this joint study was signed between the Government of India and the World Health Organization in 1956. The Government of Madras are also co-operating in the execution of tho Project by way of providing part of the staff, office equipment, accommodation, etc.

Aims and Objects

The study has been designed to yield information on the following points :—

1. The relative merits of domiciliary and sanatorium chemotherapy.

2. The efficacy of several different regimes of domiciliary chemotherapy in comparion with each other and, if indicated, with chemotherapy in sanatorium.

3. The extent to which the infectivity of groups of domiciliary patients can be reduced by chemotherapy-

4. The prevalence of tuberculosis in family contacts at the time of diagnosis of the index case and the subsequent incidence of tuberculosis amongst them.

5. The identification and virulence of the causative organisms and their comparison with strains of tubercle from England.

6. The relationship between the pathogenicity of isoniazid-resistant strains of tubercle bacilli to the guinea pig and their ability to cause relapse in the patient and to infect contacts.

7. Some of the methodological factors involved in the mass application of chemotherapy (e.g. cooperation of patients and methods of sputum collection).

The project began in the middle of 1956 and it is scheduled to continue until August, 1961. The original time table has essentially been followed and the duration of the project will depend on the length of the follow-up desired.

The Unit has steadily grown and there are now over 600 patients under treatment and over 2000 contacts, the majority under regular observation.

During the period 193 patients admitted in the previous year, completed a year of treatment according to the home or sanatorium schedules. The last patients completed the year in September, 1958.

There will inevitably be some delay with the bacteriological results since it takes two months after a culture is set up to obtain a negative result and there will also be sensitivity tests on the strains from patients still positive at 12 months.

A second investigation is in progress to compare different forms of chemotherapy for a year in domiciliary patients. During the year, over 270 patients have been admitted for this purpose. The total number will increase until some 360 are under treatment by about the end of 1958.

When patients complete a year of treatment they either receive a second year of chemotherapy or else are kept under observation without further specific chemotherapy. In this way the incidence of relapse and its possible prevention by prolonging chemotherapy are being studied. Over 170 patients are already in this second year study and some hav completed two years.

It is intended in the coming year to study the effect of a third year of chemotherapy in patients who have already had two years of uninterrupted chemotherapy to see whether relapse occurs even after two years and whether a third year of treatment can prevent it.

The contacts of every patient admitted to treatment are submitted to radiological examination and Mantoux testing, before the patient commences treatment. As far as possible all are followed up regularly in order to study the attack rate of tuberculosis in contacts in relation to the different treatment schedules adopted for the index case. It should, for example, be possible to compare the number of cases of tuberculosis which arise in contacts of domiciliary patients with those that arise in contacts of sanatorium patients. This will give an indication of the risk to which the contacts are exposed by treating the index case at home.

A dietary investigation is in progress with the collaboration of the I. C. M. R., Nutrition Research Laboratories, Coonoor (Now in Hyderabad). The object is to compare the diet of the sanatorium and domiciliary groups of patients. From the information which is being collected it should also be possible to assess the part that diet plays in the attainment of quiescence when treatment is given with standard chemotherapy.

Studies on the cooperation of patients receiving different forms of chemotherapy at home are continuing. The first report on the problems involved will be published shortly (Fox).

There is now a group of patients who have failed to respond to standard chemotherapy or have relapsed subsequently. These are receiving streptomycin plus pyrazinamide in order to study the possibility of attaining quiescence with a second combination when a first combination has proved inadequate.

A great deal of information has been obtained on the family structure and living conditions of the patients. These sociological factors are at present being codified for patients in the home and sanatorium

study. The report dealing with two aspects will document the living conditions of permanent residents of Madras in the lower income groups who are suffering from tuberculosis, and the environment in which domiciliary treatment is being undertaken.

The laboratory is undertaking approximately 1200 cultures and 300 sensitivity tests a month along with a number of tests to identify the causative organisms, and establish the virulence of strains before the commencement and during the course of treatment.

A simple, sensitive and reliable test for isoniazid in the urine has been developed and is used now routinely.

The introduction of virulence test has been a major advance in the last year and parallel studies of British and Indian strains of tubercle bacilli have been undertaken in Madras and in Dr. Mitchison's laboratory in England. These should yield valuable information on the relative virulence of pretreatment strains in the two countries.

When enough information has accumulated it should be possible to relate the virulence of the pretreatment strains to the type of disease, the response to treatment, and possibily to relapse.

A comparison has been made of the diagnostic value of sputum specimens collected on the spot in the clinic and those collected at home over a period of a number of hours.

A study is being undertaken to investigate the rate of metabolism of isoniazid in Indian patients. It is hoped to find out how the speed of metabolism influences the success or failure of chemotherapy.

A comparison of fluorescence microscopy and the Ziehl-Neelson methods of examining direct smears of sputum has been undertaken.

A comparison of standard Lowenstein-Jensen medium with the same medium containing para-aminobenzoic acid or catalase has been undertaken.

A comparison of standard Lowenstein-Jensen with Middlebrook 7H H10 medium and Sulla medium has been undertaken.

A study to investigate the increase in numbers of positive cultures which can be obtained if incubation is prolonged from eight weeks to sixteen weeks has been undertaken.

97. Enquiry on the cultural characters and pathogenicity of chromogenic acid fast bacilli and saprophytic acid fast bacilli and atypical strains of mycobacterium tuberculos s under Dr. Balbir Singh at the Irwin Hospital, New Delhi.

During the period under report, observations previously made were extended. The undermentioned material was screened with a view to select strains for study of pathogenicity in guinea-pigs.

(') Forty two specimens of smear-positive sputum, collected from untreated cases of tuberculosis were investigated by inoculating concentrates of 5 c.c. each directly into guinea-pigs with a view to make a tentative decision about the degree of pathogenicity of the strains isolated from them. They were considered "probably virulent" or 'probably attenuated' on the basis of whether Feldman indices for gross pathology observed in guinea-pigs were above or below 90 (the maximam being 100). This arbitrary grouping for the purpose of the present study was based on the observation made last year, that most strains isolated from sputum, which produced 90 to 100 Feldman index were comparable with the standard strain H 37 Rv in their pathogenicity. Fourteen strains fell into the group 'probably virulent' and the others were considered probably attenuated. Two strains of the former group and 14 of the latter were selected for the study of pathogenicity.

(ii) One hundred thirty strains of acid-fast bacilli isolated from sputum of untreated cases (including the 42 mentioned above) were :—

(a) Investigated for their ability to grow on nutrient agar at 37°C. & 22°C. respectively. Six strains that showed profuse growth on nutrient agar at 22°C. on incubation for 7 days were selected for study of their virulence for guinea-pigs.

(b) Exposed to day-light for 4 days. Four scotochromogens and 6 strains that developed slightly more pigment on exposed to light for 6 days then they had before this increase in pigmentation, however, was not such as to classity them with Runyov's pholochromogems were detected. All these 10 strains were picked out for investigation of pathogenicity.

(c) Investigated for their sensitivities against P.A.S., streptomycin and isoniazid. Six strains, that were found resistant to all 3 drugs were chosen for the present study. Catalase tests were done and 5 isoniazid-resistant catalase-negative strains were identified.

(iii) Two pre-treatment catalase-positive sensitive strains that were passaged through guinea-pigs 3 months ago and labelled as moderately attenuated because of their lower Feldman indices were tested 2nd time. This was done with a view to find whether passage of these strains through guinea-pigs could increase their virulence.

Although the total number of strains selected by the screening procedures mentioned above comes to 45 (including the 2 mentioned under (iii) above), the actual number investigated was only 38, because some strains were common to the groups selected by different procedures.

Results

P.A.S.—streptomycin—and I.N.H.—sensitive, catalase positive strains.

Non-pigmented (non-photochromic).—Twenty strains belonging to this group were examined. One strain showed 90, 100 and 95 Feldman indices on inoculation with 1, 1/10 and 1/100 mg. doses, respectively. This strain was obviously as virulent as the 5 reference strains (including, the standard strain H 37 Rv) because the average indices observed with the latter were 100, 100 and 100, respectively, in guinea-pigs inoculated with the same 3 doses and autopsied after the same interval.

The average indices observed with 5 strains were 90, 90 and 59 with the same doses, respectively. These 5 strains were nearly as virulent as the 5 reference strains because the Feldman indices resulting from them were only slightly lower than those recorded with the latter.

The average Feldman indices with the remaining 12 strains were 49, 39 and 28 with 1, 1/10 and 1/100 mg. doses, respectively. As the figures for the average indices with these strains were 50 per cent less than the corresponding figures observed with the reference strains, they were considered as moderately attenuated.

The 2 strains that had been previously passaged through guinea-pigs, produced average Feldman indices 15, 10 and 10 with the same 3 doses, respectively. These figures were in fact even less than those recorded in the test for pathogenicity done previously. Thus there was no evidence of increase in virulence for guinea-pigs after passage of these two moderately attenuated strains through the animals.

The strains (showing increase of pigment on exposure to light for 6 days).—Five strains were investigated. The average Feldman indices observed with them were 28, 25 and 15 with 1, 1/10 and 1/100 mg. doses respectively. As these figures were 70 per cent less than the corresponding ones observed with the reference strains, these strains were moderately attenuated like the 12 non-pigmented strains mentioned above.

The catalase-positive, sensitive strains could be grouped into 4 types so far as their degree of attenuation was concerned.

(i) Moderately attenuated with regression of the disease if guinea-pigs were allowed to live for 70 days.

(ii) Moderately attenuated but no regression of the disease if guinea-pigs were allowed to live for 70 days.

(iii) Slightly attenuated. This is obvious only when the dose is as low as 1/100 mg. moist weight of the organisms.

(iv) Virulent like the standard strain H 37 Rv.

P-A.S.–, streptomycin–, and isoniazid-resistant, catalase-positive strains growing on L.J. slopes at 37°C. and showing no growth on nutrient agar.

Two strains were investigated. One was non-pigmented, the other however, developed pigment on exposure to light for 4 days. Feldman indices observed on 70 day of inoculation were nil, 10 and nil with 1, 1/10 and 1/100 mg. doses, respectively, of the former, and 20, 20 and 30 with the same doses of the latter. The former was highly attenuated in virulence for guinea-pigs where as the latter was moderately attenuated.

P.A.S.–, Streptomycin– and isoniazid–resistant, catalase-positive strains showing profuse growth on nutrient agar at 37°C. or 22°C.

Scotochromogens .—Four strains were investigated. Enlargement of regional glands was recorded. No histological or cultural evidence of disease, however, was detected in these glands.

Non-pigmented (non-photochromic).—2 such strains were investigated Feldman indices with 1 mg. dose of one of these strains was 10. Histological evidence of disease was present, in the regional glands. The other strain, however, caused no evidence of disease on inoculation of the same dose of the organisms.

Probably, both the scotochromogens and the non-photochromic strains were acid-fast saprophytes. Further, more direct inoculation of sputum concentrates into guinea-pigs revealed that 2 specimens from which scotochromogens were cultured and one, from which non-photochromic strain had been cultured, carried strains of *Myco. tuberculosis* that were catalase-positive and were sensitive to P.A.S., streptomycin and isoniazid and were moderately attenuated in virulence for guinea-pigs.

Isoniazid—resistant, catalase-negative strains.

Five strains of this kind were investigated. Three were moderately attenuated. Two were highly attenuated as they produced enlargement of regional glands only on inoculation of 1 mg. moist weight of the organisms.

97. Enquiry into the susceptibility of guinea-pigs from different parts of India to infection with mycobacterium tuberculosis under Dr. R. K. Goyal at the S. M. S. Medical College, Jaipur.

The work reported earlier, showed that the individual guinea-pigs varied markedly in their resistance to tuberculosis. It was, however, not elicited whether the guinea-pigs from different parts of India differed markedly in their resistance to tuberculosis. In this investigation, in addition to testing the guinea-pigs from different parts of the country, it was also considered desirable to test the susceptibility of the animals derived from an English strain. The albino guinea-pigs reared at the King Institute, Guindy, Madras, had been originally obtained from England, hence these were also used in the experiments. In the course of this study, the suggestion that the susceptible guinea-pigs have more than 4 per cent eosinophiles or more than 10 per cent monocytes of the total W. B. Cs. (Kobe, J. Med. Sciences, 1954) has also been tested. The work already reported has shown that the weight of the guinea-pig does not play an important role in the susceptibility to tuberculosis, hence no particular attention was paid to the weight of the animals in the present investigations. The results are as follows :—

EXPERIMENT I :

The guinea-pigs from Madras (English strain-II) and Haffkine Institute, Bombay, were used in this experiment. The guinea-pigs possessing less than 10 per cent monocytes, or less than 4 per cent eosinophiles were not always less susceptible than the other guinea-pigs possessing a higher percentage of these leucocytes. The Madras guinea-pigs derived from the English strain varied to some extent in their resistance to tuberculosis, but appeared to be more uniform in their resistance than Haffkine guinea-pigs.

EXPERIMENT II :

Only seven guinea-pigs obtained from Patna could be used in this experiment. There was a marked variation in their resistance to tuberculosis. It may, however, be mentioned that these guinea-pigs had been obtained from private dealers. The differential leucocyte count did not appear to be of value in the determination of their resistance.

EXPERIMENT III :

The guinea-pigs from the following sources were used in this experiment :—Patna-20 guinea-pigs, Calcutta-36 guinea-pigs, Madras (English strain)-10 guinea-pigs. The Patna and Calcutta guinea-pigs obtained from local dealers showed more marked variation in their resistance to tuberculosis than Guindy guinea-pigs derived from the English strain. The differential leucocyte count was not found to be of value in determining the resistance of guinea-pigs.

During the course of cultures of the organs of guinea-pigs for tubercle bacilli, fungi were commonly isolated. It was not clear whether the fungi were ærial contaminants or were present in the organs of the guinea-pigs. In a preliminary series of experiments, the following fungi have been isolated from culture tubes inoculated with the organs of guinea-pigs or from Sabourand's medium exposed to the air in Petri dishes :—Mycoderma, monotospora, mucor, hormodendrum, trichoderma, hemispora, torula, aspergillus and penicillium.

98. Tuberculosis field research project, under Dr. Frimodt Moller, Madanapalli.

I. Field Work During 1957-58.

a) Madanapalle Rural Population

Since 1950 a study of the tuberculosis epidemiology and the possibility of reducing the prevalence of tuberculosis by repeated systematic examinations by mass miniature radiography and isolation of infectious cases have been carried out in about 200 villages near Madanapalle with a population of about 40,000, besides the town population of about 20,000. The last examination by M. M. X-ray took place in 1954-55 after which the mobile x-ray unit was engaged in a sample survey of Andhra and Mysore States (and North Arcot District) under the I. C. M. R. National Tuberculosis Survey. When this was completed in April 1957 a new survey of the local village population was undertaken. The survey of the villages has been completed while the examination of the town population is still proceeding. In 194 villages with a total population of 40,854, there were 35,442 persons eligible for x-ray (after excluding children below 5 years). Eighty nine per cent of these were x-rayed and owing to the presence of suspicious x-ray shadows, 1.7 per cent were selected for bacteriological examination. In 2.6 cases per 1000 persons x-rayed tubercle bacilli were demonstrated. Of the Town population 17,413 are eligible for x-ray ; so far, 83 per cent have been x-rayed but the examination is not yet completed.

(b) Madanapalle Local—Inaccessible Villages

When the village survey was begun in 1950 there were 341 villages within about ten miles of Madanapalle. It was not possible for our mobile x-ray unit to reach 141 of these villages and hamlets. As a new portable x-ray set was lent to us in September 1957 by the W. H. O. it was decided to examine a sample of these villages hitherto inaccessible. A random sample of 50 villages were drawn, and during the hot season in June 1958 when the fields were bare and the soil hard 26 villages with a total population 3352 were examined; the remaining 24 villages and hamlets were lying so isolated and remote from ordinary cart tracks that they could not be done unless the equipment was carried on head loads or by donkey. Among the persons living in the 26 villages 90 per cent were examined (excluding the small children) and 1.2 per 1000 x-rayed had active pulmonary tuberculosis.

(c) Inaccessible villages, under the ICMR National Tuberculosis Survey—Madanapalli Zone.

When the sample survey was undertaken in 1955 it was found that among 180 villages drawn at random from the official Census lists of India, only 56 per cent were accessible to our mobile x-ray unit. When the new apparatus mentioned above became available, it was decided to examine a random sample of these "inaccessible" villages. Accordingly, fifteen villages scattered throughout Mysore, Andhra Pradesh (Southern part) and in the North Arcot district of Madras State were selected having a total population of 8,636. Of the 15 villages, 2 had to be dropped due to

certain insurmountable difficulties. That leaves 13 villages, completed. Of a total of 7,804 persons, 6,507 were eligible for x-ray and 92 per cent of them were examined; 4.8 per cent of these were selected for bacteriology and 10 per cent of the specimens and cultures so far examined have shown presence of tubercle bacilli, i. e. a rate of 4.7 per 1000 persons x-rayed.

(d) BCG-Control Trial among students at the Christian Medical College, Vellore.

Since the academic year 1950-51 till 1957-58 a total of 2395 medical and nursing students and staff have been examined by tuberculin testing and M. M. x-ray and followed up; 2023 were considered to be reactors to tuberculin, of the non-reactors 204 were BCG vaccinated and 168 left unvaccinated. The investigation is continuing.

II. Analysis of Previous Work.

(a) From 1950 to 1955 four rounds of tuberculin testing and x-ray examination were done in the Madanapalle village population. From 1956-57 the findings have been analysed and the results obtained have been embodied in a comprehensive report which in September 1958, has been submitted to the Government of India and the W. H. O., the two bodies which together with the U. M. T. Sanatorium have sponsored the investigation. The report is expected to be published in 1959 in the W. H. O. Bulletin.

(b) All the data obtained under the ICMR National Tuberculosis Survey by the six different centres have been analysed at the statistical department of the Madanapalli Field Research Station.

99. Enquiry on Bronchoscopic Studies in Cases of Pulmonary Tuberculosis under Dr. R. N. Tandon at the Kasturba T. B. Clinic and Hospital, King George's Medical College, Lucknow.

The study consists of 166 cases (116 male and 50 females) of pulmonary tuberculosis proved on clinical, bacteriological and radiological investigations. The cases were taken as they came in, without any particular selection. At the time of admission 103 cases (62.1 per cent) had stage III, 46 (27.7 per cent) Stage II and 17 (10.2 per cent) Stage I of parenchymal disease (Classification of Indian Tuberculosis Association, 1948). In 74 cases (44.6 per cent) the sputum was positive for A.F.B.

Results

(1) The incidence of endobronchial disease in the present series was 46.9 per cent. In female it was a little higher (52 per cent) than in males (44.8 per cent).

(2) The bronchial aspirate showed presence of bacilli in 81 cases while sputum was positive in 69 cases. On the other hand, in 5 cases the sputum examination showed A.F.B. while bronchial aspirate was negative.

(3) The clinical evidence of endobronchial involvement was present in 41 cases and out of these bronchoscopy showed presece of disease in only 32 cases (78 per cent). Out of 78 cases who had positive bronchoscopic findings the clinical evidence of disease was present in 32 cases (41 per cent) only. Twenty two per cent of cases with negative bronchoscopic findings showed clinical evidence of, disease while the comparative figure of B. T. A. (1953) is 14 per cent. Other workers who have investigated this subject, have come to the conclusion that the clinical assessment is not a reliable guide to the presence of endobronchial disease.

(4) Out of the 50 cases who were followed up, 78 per cent showed improvement (40 per cent having completely healed), 4 per cent became worse and 18 per cent remained stationary. The comparative figures of B.M.R.C. (1951) are 68 per cent completely healed, 20 per cent improved, 9 per cent remained stationary and 3 per cent became worse. The results were not so good in this series probably because most of th e cases were in advanced stage and many were resistant to drugs. Best results were observed in cases that were not treated earlier and in those with Stage I extant of paranchymal disease.

(5) Complications were noted in 9.6 per cent of cases only. Most of the complications were insignificant except in 1 case in which spread of disease occured. This case inhaled vomitus while bronchoscopy was being performed and few days later the radiological examination showed spread of disease to healthy areas.

100. Diagnosis of active primary pulmonary tuberculosis in children with old tuberculin of varying dilutions by intracutaneous multiple puncture method under Dr. Saktipade Bhattacharjee at the Nilratan Sircar Medical College and Hospital, Calcutta.

AIMS AND OBJECTS OF THE WORK.

A. To find out the correlation between the different stages of primary pulmonary tuberculosis in children below the age of 7 years and the degree of sensitivity to different dilutions of old tuberculin using Mantoux method.

B. To find out the influence of therapy using different groups of anti-tuberculous drugs (drugs supplied by the institution) on the degree of sensitivity to different dilutions of old tuberculin using Mantoux method.

BRIEF SUMMARY OF THE WORK.

Five hundred cases below 7 years of age were included in the investigation. Tuberculin tests were carried out in all these cases with old tuberculin solutions of varying dilutions starting from one in million working up to a dilution which gave positive reaction. Injections of different strength of old tuberculin were given every 4th day on the alternate forearm to the negative reactors. Two hundred and thirty cases showed positive reaction (definite induration of a diameter of 6 m.m. or more).

Laboratory, clinical and radiological examinations were carried out in these cases to establish diagnosis. The economic status and environment were also studied with a search for the source of injection.

A. Radiologically positive cases were grouped as follows :

Group I : Active primary pulmonory tuberculosis–presence of glandular and/or lung component.—Sixty six per cent of cases belonging to this group showed positive reaction with old tuberculin in a dilution of 1 in 1,000,000, 16 per cent in 1 in 100,000, 5 per cent in 1 in 10,000 and the rest showed positive reaction in dilutions between 1 in 1000 and 1 in 10. Assessed clinically and by laboratory aid, particularly by E.S.R. it could be seen that in the cases with positive reaction in 1 in million dilution the disease was more active, and required anti-tuberculous therapy. Amajority of the other cases showing positive reaction between 1 in 100,000 and 1 in 10 dilutions showed gradual improvement without administration of anti-tuberculous drugs as assessed by periodical check up (radiological, clinical and laboratory findings).

Group II : Early progressive primary pulmonary tubercnlosis .—Evidence of progressive glandular and/or of parenchymal lesion. Eighty six 86 per cent of the cases of this group showed positive reaction with 1 in 1,000,000 ;

9 per cent with 1 in 100,000; 2 par cent with 1 in 10,000 dilutions, and 3 per cent showed positive reaction between 1 in 1000 and 1 in 10 dilutions.

In this group most of the patients showed positive reaction with 1 in 1,000,000 dilution. The cases showing positive reaction with stronger dilutions of old tuberculin gave history of diseases such as measles, whooping cough; typhoid fever, etc., which might have lowered the resistance and some of them did show very low general condition assessed clinically.

Group III ; Progression of stabilized primary complex at a later date.—All the cases of this group showed positive reaction with 1 in 1,000,000 dilution.

Group IV : Evidence of hæmic and lymphatic spread.—Seventy per cent of the cases of this group showed positive reaction with 1 in million dilution, 8 per cent with 1 in 100,000. 10 per cent with 1 in 10,000 and 12 per cent with dilutions from 1 in 1000 to 1 in 10.

Group V : Healed primary pulmonary tuberculosis.—Disappearance and/or calcification of components-parenchymal and glandular.—Sixtyeight per cent of the cases of this group showed positive reaction with 1 in million dilution, 8 per cent with 1 in 100,000, 8 per cent with 1 in 10,000, 10 per cent with 1 in 1,000 and 6 per cent with dilutions between 1 in 1000 and 1 in 10.

*B. **Follou-up:***

Depending on the anti-tuberculous drugs administered the patients were grouped as below:—

Group I. Cases treated with isoniazid and P.A.S.

Group II. Cases treated with streptomycin and P.A.S.

Group III. Cases treated with streptomycin isoniazid.

During treatment, routine checking was carried out by E.S R skiagram and graded tuberculin test at intervals of three months.

Group I. (Isoniazid and P.A.S.).

Sixtynine per cent of the cases of this group showed somewhat less severe reaction with 1 in million dilution as compared to the results of free treatment testing 5 per cent of the cases were converted from one in million to 1 in 100,000 (i. e. they showed negative reaction or the diameter of the reaction was less than 6 m.m. with 1 in 1,000,000 dilution but positive with 1 in 100,000 dilution). Five per cent of the cases were converted from 1 in million to 1 in 10,000, another five per cent from 1 in million to 1 in 1000, and again an equal number from 1 in million to a dilution of 1 in 100 or 1 in 10. One per cent of cases gave negative reaction with all dilutions. There was no change in the type of reaction in about 50 per cent of cases while in an equal proportion of them the reaction showed an increased intensity.

Group II. (Streptomycin and P.A.S.).

Most cases of this group showed decreased reaction 1 in 1,000,000 dilution.

Group III. (Streptomycin aud Isoniazid).

Most cases in this group also showed a reduction in the intensity of reaction with 1 in 1,000,000 dilution.

Sensitivity decreased after the administration of anti-tuberculous drugs and a few in the series showed a negative reaction even with 1 in 10 dilution.

Cases showing unaltered or increased sensitivity to tuberculin were usually those who had an attack of a debilitating disease, or could not afford good diet and/or specific drugs for some unavoidable reasons. Some of these patients, after their admission to the indoor department, were provided with nutritious diet and adequate specific treatment on admission to the hospital, showed reduced sensitivity to tuberculin.

C. A group of mantoux positive cases were kept under observation as they showed no radiological findings in the beginning. Some of these patients developed radiological lesions during the follow-up. They were grouped according to the radiological findings and anti-tuberculous drugs were administered with better results.

101. Enquiry into morbid anatomy and bacteriology of resected lungs in pulmonary tuberculosis under Dr. D. Barua at the Institute of Post-Graduate Medical Education and Research, Calcutta.

Resected tuberculous lungs specimens from 70 patients were taken up for histo-bacteriological investigation. Most of these patients came from lower middle class families of poor socio-economic status. Nine out of these 70 cases were of less than one year's duration and 16 were of more than 5 years duration. All of them were treated by various combinations of antituberculous drugs by private practitioners without strict supervision. The material of this study was thus different from those of similar investigations of other countries were the therapeutic regime was under rigid control with the additional advantages of bed-rest, good food, etc.

The specimens were collected with all aseptic precautions. Altogether 285 lesions of different anatomical types viz., cavernous, caseous, paltaceous, inspissated and calcified were studied. The materials from different lesions were examined microscopically for a.f.b., were cultured in 3 tubes of L.J. media and were inoculated into 2 guinea-pigs at the same time to look for the presence of the organisms, their viability and their virulence. Those organisms which grew in culture but failed to cause progressive disease in guinea pigs were injected (1 mg. wet weight) into guinea-pigs again. All the viable bacilli were tested for their drug sensitivity pattern to Sm. PAS & INH by inoculating the organisms into L.J. media containing the drugs in different concentrations. H37RV strain was used throughout the study to test the batches media prepared and as control for the drug sensitivity test and for guinea-pig inoculation tests.

Test for production of catalase and neutral red test were also carried out in an attempt to find correlation with the INH resistance and virulence.

No a.f.b. could be demonstrated in 9 out of these 70 cases. Visible non-visible bacilli were found in 33 (47.1 per crnt) cases and viable bacilli could be isolated from 28 (40.0 per cent) cases. Seyen out of these 28 strains were avirulent to guinea-pigs. Viable-virulent organisms were frequently found in pultaceous lesions. No secondary organisms including Fungi could be isolated from the lesions.

In an attempt to correlate bacteriological status with the duration of illness and treatment, viable bacilli were found more often in the lesions of cases of more than one year duration. Among the cases of longer duration, there was no direct correlation with the amount of total drugs taken by them. Almost all the cases of the groups of longer duration of this series had fairly large doses of all the three drugs or two of them but the irregularities and interruptions could not be avoided which would probably explain the failure of such large doses to eradicate the organisms.

Of 62 viable strains isolated from different lesions, 53.2 per cent were Sm-resistant, 12.9 per cent PAS-resistant and 53.2 par cent INH-resistant. Isolated PAS-resistant strains were not encountered.

Sixteen strains were found to be resistant to 100 meg of INH, catalase, negative and avirulent to guinea-pigs. They were, however, neutral red positive.

Sections were taken from the representative lesions of different anatomical types and from their different parts. They were studied carefully for the histological evidences of activity or regression of lesions. The cavernous lesions were considered to be active lesions where there were tuberculous follicles in their walls, caseation in the linings, active cellular infiltrates in the surrounding parenchyma with or without evidences of disease in the draining bronchi. Active parenchymal disease were considered to be present when there were lobular and caseous pneumonia, perifocal reaction was present and actual caseation extended into surrounding tissues.

Complete absence of caseation in the cavitary lesions, absence of caseous pneumonia and perifocal reaction together with evidences of fibrosis and a modified cellular response in and around other lesions were regarded as evidences of morphological regression. Such regressive changes were frequently seen in the lesions which were bacteriologically sterile or which visible non-visible bacilli were demonstrated.

The evidences of activity were, however, almost invariably present in the lesions with viable-virrulent and viable-avirulent bacilli. No correlation could be found between the virulence of the organism to the guinea-pigs and the histological evidences of activity of the lesions. Histological evidence of activity has also been noticed around some lesions which were sterile or had visible non-viable organisms. Such findings seem to favour the views of some workers who prefer to consider the visible non-viable organisms as potentially Pathogenic microbes. The classification of non-viable organism as a seperate group by judging their pathogenicity on guinea-pigs and growth on L.J. media may not be able to reflect the effect of the bacteria on the human host.

The results of this study indicate that the present system of treatment of pulmonary tuberculosis without strict supervision and with no ancillary measures is not likely to yield a therapeutic cure. The persistence of viable bacilli and histological evidences of activity of the lesions after years of treatment and after such large total dosage of the drugs indicate that the strict supervision is required to enforce regularity in the regimes. These incompletely treated cases are likely to play a very significant role in the epidemiology of the disease.

102. Pharmacological studies on a new anti-tubercular antibiotic under Dr. S. Chandrasekhar at the Vallabhbhai Patel Chest Instt., Delhi.

Four or 5 trails had to be made before the medium could be properly sterlized. Nutrient Broth (pH 7.4) which was tried as the fermenting medium foamed, in spite of the addition of an antifoaming agent. Therefore, the medium was changed-Glucose and Aspragin Broth (PH7.4) was used with an antifoaming agent. Since this broth does not contain peptone foaming was very little. This time no contamination occurred and the medium (10 liters) could be successfully fermented. All of it could not be extracted because ethylene dichloride was not available.

It was, therefore, decided to substitute another solvent for ethylene dichloride, if possible. In a later series of experiments 10, 15 or even 20 litres of the broth has been fermented. The crude antibiotic was extracted with ethyl acetate, as ethylene dichloride was not available. Yields were slightly better in this case. Chemical purification was tried on the crude antibiotic obtained by vacuum distillation of the ethyl acetate extract. The active substance is phenotic in nature, probably a catechol. It could be separated into two substances, one white, wax like substance soluble in petroleum either and insoluble in methanol. Another red oil-like substance soluble in methanol and insoluble in petroleum ether. However, whenever these two were separated the activity was lost, the change being irreversible.

A sodium salt of crude substance prepared by shaking the ethyl acetate extract with Toxicity studies in mice indicated that 100 mg./kg. body-weight by intravenous route and 125 mg./kg. body weight by subcutaneous route were well tolerated by these animals.

VENEREAL DISEASES

103. Enquiry on treponemal antigen tests for syphilis under Dr. C.W. Chacko, Venereal Diseases Laboratory, Government General Hospital, Madras.

Aim and scope of the enquiry has been to reproduce and evaluate the reported specific value of the various new serological tests for syphilis, using antigens of virulent treponemal origin, one by one, by comparing them with standard tests in vogue (VDRL) using non-specific tissue extract antigen, to carry out these tests in various clinical conditions, and to establish them, at least, as reference tests for syphilis in the country.

During the period under report, a total of 399 specimens of sera were investigated with T. Pallidum Immune Adherence test (T.P.I.A). These included 146 specimens of sera from various stages presumably syphilis cases of diagnosed, clinically. This clinical diagnosis was confirmed in 143 cases by the TPIA test, compared with 140 by the VDRL test for syphilis. In 3 cases, one each of tabes dorsalis, Congenital syphilis with only Hutchinson teeth, and Interstitial keratitis, the TPIA test had apparently confirmed the clinical diagnosis while the VDRL test did not. This emphasises the dependable sensitivity of the TPIA test. There were 3 negative agreements between the two tests in this group and they were all clinically "primary syphilis" cases where probably the antibodies concerned had not reached the threshhold level for detection.

In the non-syphilitic group of 116 apparenty normal persons, there had been 100 per cent agreement between the two tests indicating their possible specific value.

However, the two tests disagreed in 16 out of 108 non-syphilitic abnormals. In these 16 cases of miscellaneous clinical categories, the exclusion of syphilitic infection clinically has been apparently supported by the TPIA test. Since these cases have given position VDRL reaction they must be considered "biologically false positive reactors" While this again highlights the specific value of the TPIA test as a "Verification or Reference test", there is need for a more extensive investigation of this type of sera before definite conclusions are drawn. This is particularly so in view of the fact that in 7 out of 108 non-syphilitic abnormals both the tests have been positive, throwing doubt on the validity of excluding clinically, past or present syphilitic infection in them.

A quantittaive technique has been performed on 50 qualitatively TPIA positive sera, with parallel standard quantitative VDRL tests. The results seem to show similar trends but the technique need critical analysis before it may be considered "a standard method" for practical use.

The TPIA antigen preparation and the test technique as carried out now, are difficult and expensive to be performed. for what they are worth, as a routine in all laboratories. A test called Reiter Protein Complement Fixation (RPCF) test for syphilis has been more recently described and reported on favourably for its specific value (Rein, C.R. 1957, Jour. Invest. Dermat. 28, 459). It has the advantage that the antigen is more conveniently prepared in greater quantity from the cultivated Renter treponeme.

104. Enquiry on the isolation and establishment of Virus of lymphogra, nuloma venereum (LGV) in embryonated chicken egg and the preparation of Frei antigen from it under Dr. C. W. Chacko- Venereal Diseases Laboratory, Government General Hospital, Madras.

The enquiry was started on 1st April 1956. Up to the 30th Sept. 1958, 39 specimens of human LGV bubo pus, were obtained.

The Frei antigen from human pus was prepared directly from 23 of the 39 specimens, according to Frie's original technique, 18 samples of the antigen, so prepared, proved potent on clinical trial. The quantity of antigen produced was so limited that it was adequate only for local use.

Attempts to isolate a local strain of the virus of LGV from out of the 39 specimens of pus, primarily, either in the embryonated hick yolk sac, or intracerebrally in Swiss white mice, according to the widely adopted techniques described earlier, have not been successful.

As an alternative to the source of the virus from bubo pus, in which the viability of the virus may have been questionable, the whole bobu gland at an earlier stage of infection without fluctuation, was also used. Three specimens of glands were used in this experiment without any more success. It was observed in the course of this experiment that one of the whole glands material killed 50 per cent of the mice, specific-ally after 48 hours. However, the virus possibly isolated in the brain of the mouse could not be maintained successfully as the virus was lost af-ter about 6 to 9 sub passages. Even the possible primary isolation in the brain was not conclusively proved by demonstrating elementary or initial bodies of the virus in impression smears of the meninges and brain, stained with Casteneda and Gram stains.

Duplication of the experiments to isolate and preserve the virus from one of the gland specimens used above, at the Virus Laboratory, Pasteur Institue, Coonoor, also did not meet with any success.

Efforts to revive and establish a standard laboratory strain of the virus of LGV were also made at the same time. To this end, lyophilised samples (9 in all) of the egg-adapted J.H. strain from Dr. Barwell of London Hospital and the KAM strain from Dr. Lepine of Pasteur Institute, Pariswere transported to Madras, by air. Attempts to revive them, in the chick yolk sac according to their instructions, in 5 separate trials have also not been successful.

It is rather surprising, that while it has been possible to establish and maintain the closely-related species, like psittacosis virus under the same laboratory facilities, it has not been possible to isolate the LVG virus. Probably, the latter is not easily adapted to the Laboratory conditions.

Antigenically related and closely resembling morphologically, as these two viruses are, a trial preparation of skin antigen from the psittacosis virus, similar to LGV Frei antigen of yolk sac origin, was made by Grace's method (1940). Preliminary clinical trials for its diagnostic value by parallel skin tests with it Frei antigen of human pus origin were made on 50 cases. They agreed in positive reactions in 12 cases of known LGV, in negative reactions in 10 of 11 cases of normals and in 13 out of 27 VD cases other than LGV. There were 10 inconclusive results for the psittacosis antigen.

There is scope for more extensive trials with the psittacosis skin antigen on the analogy of the reported value of the complement fixing antigen of Psittacosis orgin, in the diagnosis of LGV.

However, in view of the shortage of supply of LGV Frei antigen of local or foreign origin to meet the great demand in the VD clinics in India, it is planned to persevere with the trials to establish a strain of LGV virus in the laboratory, seeking the active collaboration of experts from India and abroad.

105. Co-operative study of the patients of Mental Hospital, Madras, with reference to the role of syphilis as direct or indirect factor in the causation of mental diseases under Dr. R. V. Rajam at the Govt. General Hospital, Madras.

Between 1st May 1958 and 30th September 1958, 276 patients (male) who sought admission into or advice from the Govt. Mental Hospital, Madras, and the Psychiatric out-patient clinic of Govt. General Hospital, Madras, were screened and subjected to the following investigations.

1. Psychiatric analysis.
2. Clinical and neurological examination.
3. Examination of the blood and cerebro spinal fluid.
4. Roentgenological examination of the cardioaortic system.
5. Ocular examination.
6. Brain biopsies of patients with syphilitic psychosis.

The diagnosis of General paresis in 14 patients and syphilitic psychosis of non-paretic type in 1 patient was established on the basis of the psychiatric, neurological and serological findings. The duration of syphilitic infection could not be elicited in these cases. The duration of the mental disorder was obtainable only in 9 cases that varied from 6 to 18 months. The average age of the patients with syphilitic disease was 41 years, the youngest being 30 years and the oldest 58 years.

Investigations of the social and occupational status of the patients of syphilitic psychosis showed that they were mostly of the lower middle class consisting of artisans, domestic workers, petty salesmen etc., belonging to the non-intellectual type, except 2 patients—one a businessman and the other an accountant who may be said to belong to the intellectual middle class.

Brain biopsies in 5 patients showed the characteristic histological features of General paresis with the pathognomonic positive Prussian blue reaction. Among the 14 General paretics, 7 were of the Simple Dementing type and 3 of the Grandiose type. The Agitated type, Korsakow's Syndrome type, Lissauer type, Acute galloping type shared one each amongst the rest. There was one case of non-paretic syphilitic dementia due to diffuse meningo-vascular neuro-syphilis.

Patients with syphilitic psychosis in the series were treated with Penicillin alone, Penicillin with pyrexia and Penicillin with cortico steroids. Two patients in the series died 2 weeks after the completion of therapy, one of them belonged to the acute galloping type and the other developed status eqilepticus. The remaining patients are still under observation.

It is too early for post-treatment assessment, although 3 patients showed improvement psychiatrically. The oft quoted statement that parenchymatous neuro-syphilis, particularly the paretic type is a rare disorder in the tropics is disproved even in this limited study.

Two observations are worth recording in this study.

1. Treponemin skin test which is claimed to be positive in all cases of late syphilis is found to be completely negative in the series under the present study.

2. There were 25 patients, whose blood showed positive reaction but whose spinal fluid was negative, exhibiting the varying types of psychiatric syndromes mostly consisting of the Manic Depressive Psychosis and Schizophrenic types.

The relation of the latent syphilitic infection to the psychiatric status is not clear and will be investigated further.

106. Enquiry into liabilities to venereal infection by migration and transference of rural population to industrialized urban centres under Dr. H. I. Jhala, Director, Haffkine Institute, Bombay.

It was decided to start the work with the workers of Swadeshi Mills, Kurla. Blood samples were primarily examined for Kahn and V.D.R.L. tests. It was decided to give possible additional information, e.g. Hb per cent blood count in abnormal cases blood grouping, etc. Examination of cases with positive or doubtful serology was started at the Factory Dispensary, Kurla, twice a week. So far 57 sero position cases were examined of which 27 were referred for treatment. None showed evidence of early infectious syphilis. Only one case with sores on the phallus was detected. Twenty contacts were traced so far. The social worker contacted 750 workers and filled in history sheets.

Laboratory investigations on blood samples were carried out in the Dept. of Clinical Pathology, Haffkine Institute. The report on various blood tests done from 14th June to 30th Sept. is given below :—

Total No. of workers in Swadeshi Mills	..	5509
Total No. of blood samples collected	..	1043
Total No. of blood samples examined by K. T. & V.D.R.L- tests	..	1021
Total No. of reactive sera	..	70
Total No. of non-reactive sera	..	918
Total No. of weekly reacting sera	..	33
Total No. of blood samples examined for Hb per cent	..	1014
Total No. of showing Hb% less than 12.5 g. per cent	..	19
Total No. of samples examined for routine blood grouping	..	1014

Total No. of samples selected at random by the Civil Surgeon, Ratnagiri and sent here for K.T.& V.D.R.L. examination.

No. of samples received.	..	248
No. of samples found unsuitable	..	16
No. of samples examined	..	232
No. of reactive sera	..	24
No. of non-reactive sera	..	208

PLAGUE

107. Standardization of technique in the study of resistance of fleas against DDT under Dr. P. Sen at the school of Tropical Medicine, Calcutta.

Although this enquiry is meant for evolving a standard technique for testing the resistance of fleas to DDT, a few other insecticides have also been investigated.

The work so far carried out in this respect, as also the various methods and the insecticides used, are given below.

I. Insecticides in Dust Form

(a) *Test chamber* : Large-sized test tubes 5″ x 1″, or specimen jars 8″ x4½″ x 3½ used as test chambers.

(b) *Insecticides and dosages used :*

DDT—0.1 per cent, 0.5 per cent, 1 per cent

Diazinon—5 per cent, 10 per cent

(c) *Mode of application* : (i) The bottom of the test tube filled to the height of 1″ with sterilized sand mixed in bran, sawdust, softstone powder or rabbit fæces. On top of this inert material a light dressing of the insecticide was applied in dust form at the dosages mentioned above. Mouth of the tube was plugged with cotton wool.

(ii) The bottom of the jar referred to above was similarly filled to the height of 2″ from the bottom and dressed with the insecticide dust on top. Mouth of the jar was protected by fixing a gauge cloth.

(d) *Test procedure :* One hour's exposure was given in every experiment and the mortality recorded after 24 hours, the fleas being removed to the breeding jar with a caged rat after 1 hour exposure to provide an opportunity of blood-feed to the fleas. Three to six replicates, each of eight fleas, *Xenopsylla cheopis*, involving both sexes were run in each experiment.

(e) *Result:* The mortality was as follows:—96 per cent in 0.1 per cent DDT when sand particles alone was used as substratum, 87.5 per cent in 0.5 per cent DDT when sand and bran were employed as the substratumt 96 per cent in 0.5 per cent DDT by replacing bran with rabbit faeces, bu, the mortality was 77.5 per cent only at the same dosage of 0.5 per cent DDT when sawdust was used as substratum. At a dosage of 1 per cent DDT, with sand as the supporting material, the mortality was cent per cent.

The mortality rate was the lowest in sawdust, and maximal in sand grains or rabbit fæces. The LC50 when dust form was used worked out to 0.32 per cent DDT.

Diazinon as 10 per cent dusting powder when used in a substratum of sand gave a mortality of 56.6 per cent and as 5 per cent dust, the mortality was less than 50 per cent But at a higher atmospheric temperature of 109°F, 48 hour old unfed fleas showed a mortality of 75 per cent even at the last mentioned dosage of 5 per cent.

II. Insecticides in Solution

(a) *Test chamber :* The same as recommended by the WHO after Busvine and Nash. *Mode of application* of the insecticides and *test procedures* were also the same as recommended by the WHO, for testing mosquito adults.

(b) *Insecticides and dosages used :*

DDT	..	0.5 per cent, 1 per cent
BHC	...	0.05 per cent, 0.1 per cent
Malathion	..	0.1 per cent, 0.25 per cent

(c) *Method :* The exposure to the insecticide was given for an hour and 24 hours' mortality was recorded on transference of the fleas to the breeding cages as mentioned under test *procedure above.*

(d) *Results :* Computed from six replicates of eight fleas each. The mortality was 83 per cent in 1 per cent DDT, and 60 per cent in 0.5 per cent DDT. With BHC, the mortality was 83 per cent and 42 per cent in 0.1 per cent and 0.05 per cent dosages respectively. With Malathion, the mortality proved to be 89 per cent and 34 per cent, respectively in the two dosages of 0.25 per cent and 0.1 per cent.

(e) The LC50 of these insecticides was found to be as follows: DDT—0.4 per cent, BHC—0.06 per cent, and Malathion—0.15 per cent.

It will thus appear that LC50 of 0.4 per cent in DDT when used as solution was not very far from that of 0.32 per cent obtained with DDT in dust form.

108. Study of rodent species and their susceptibiliy to plague infection in the epidemic areas of Bombay State under the Director, Haffkine Institute, Bombay.

During last year rats from five different zones *Viz* . (1) Bombay, (2) Andhra, (3) Madras, (4) Assam and (5) Uttar Pradesh were received. The rats were kept under observation for a period of one week and those which were very much under-sized or otherwise unfit for experimental work were discarded. The rats were kept individually in different cages and were challenged by a test infective dose consisting of about 4000 to 8000 organisms of a fully virulent strain of *P. pestis*. The animals were observed for a period of three weeks. The animals that died during the period of observation were autopsied and death due to plague was confirmed by post mortem findings and examination of spleen smears. Along with each batch of rats, a bach of 5 mice was also put up as control. The mice were given 1/50th of the test infective dose (containing about 80 to 160 organisms) given to rats. Hundred per cent of the mice died in every batch, showing thereby that the infective dose given to the rats of each batch was fully virulent.

Conclusions :—From the mortality rates among rats, it would appear that the most valnerable zone to plague epidemics is the Assam zone, next in order being Andhra, Bombay, U.P and Madras.

The results confirm the observation of previous workers that a series of epizootics among the rats, have left behind the rats resistant to plague infection and as the percentage of resistant rats increases the places become less and less vulnerable to plague epidemics. The observations show the comparative vulnerbility of the different endemic foci to possible epidemics in future and hence are of great value to take adequate measures in time to ward off the threatened epidemics.

The work is continuing.

109. Study of development and growth of dentition of Indian Children under Dr. R. S. Nanda at the Dental College and Hospital, Lucknow.

The present study was started in April, 1957. Up to date 1000 individuals from both sexes have been examined. The examination of each individual included (i) a detailed history, (ii) a thorough clinical examination, (iii) preparation of dental casts and (iv) full mouth intra-oral radiographs.

Analysis of the data pertaining to intra-oral X-rays of the subjects in three age groups has also been started. The age groups selected were 6, 8 and 10 years, for the simple reason that maximum number of cases had been examined at these ages. The study of the X-ray was limited to include only the extent of tooth development (deciduous and permanent) and root resorption (deciduous).

Methodology

For evaluation of the stages of development, the root of a tooth was assumed to be twice the size of its crown. The tooth crown was arbitrarily divided into four equal segments and the tooth root into eight. Stage two was marked at a level when the maximum diameter of the crown formation had been completed and stage four, when the crown appeared to be completed. The root length was divided into eight equal segments. In classifying the teeth into various segments for the evaluation of the tooth formation and root resorption, the standards used by Gleiser & Hunt were taken as a general guide. The definition of different segments adopted in this study and their corresponding segments used by Gleiser & Hunt have been presented in Table I From an examination of the intra-oral radiograph the level of development of tooth formation (permanent dentition) and root resorption (deciduous dentition) for each of the teeth present in an individual were carefully recorded. Wherever there appeared to be a doubt regarding the segment to which the tooth could be assigned, the decision was always in favour of counting the tooth in the more advanced segment. In this manner charts pertaining to each tooth of permanent and deciduous dentition were prepared.

Analysis

The data have been analysed to obtain the average level of development of dentition at each age period and presented in Tables II and III separately for deciduous and permanent teeth. The maxillary and mandibular teeth were always considered separately. The data for the two sexes were also separated for six-year old children, in order to verify whether there was any significant difference in tooth formation and resorption in the sexes. These results showed some degree of sex difference in the deciduous teeth. The maximum difference was observed in the case of mandibular central incisors which showed the first stage of root resorption earlier in males compared to females. In the case of mandibular lateral incisor, however, the females appeared to have more resorption than the males. Maxillary central incisors showed more resorption than any other tooth in the arch at the age of six years and this was

TABLE I.

Stages of Development of Permanent Tooth.

Lucknow Standards	Gleiser & Hunt Standards
12 stage root canal terminally convergent—completed	15 stages
11 stage root canal terminally convergent 7/8 completed	14 stages
0 stage 3/4 completion	13 stages
9 stage 5/8 completion	12 stages
8 stage 1/2 completion	11 stages
7 stage 3/8 completion	10 stages
6 stage 1/4 completion	9 stages
5 stage Beginning of root formation or 1/8 completion	8 stages
4 stage Crown completed up to E. C. Junction	7 stages
3 stage 3/4 crown completed	6 stages
2 stage Half of crown completed	5 stages
1 stage Outline of cusps completed	1, 2, 3, 4, stages (from the appearance of 1st sign of calcification to the outline of cusp.

Resorption of Deciduous Teeth

Lucknow Standard.

8 stage—complete resorption
7 stage—7/8 resorption
6 stage—3/4 resorption
5 stage—5/8 resorption
4 stage—1/2 resorption
3 stage—3/8 resorption
2 stage—1/4 resorption
1 stage—1/8 resorption
0 stage—No resorption

TABLE II.

State of Deciduous Teeth at 6, 8 and 10 Years of Age in both the Sexes

Teeth	Extent of Resorption According to Segments					
	Maxillary			Mandibular		
	6 Years	8 Years	10 Years	6 Years	8 Years	10 Years
i_1	3.57	7.45	8.00	0.50	7.85	8.00
i_2	1.65	6.35	8.00	1.12	6.50	8.00
c	0.00	1.70	2.80	0.00	2.50	3.45
m_1	1.37	5.45	5.45	1.90	4.60	4.10
m_2	0.50	4.80	5.30	0.72	3.50	3.65

TABLE III.

State of Permanent Teeth at 6, 8 and 10 Years of Age in both the Sexes

Teeth	Extent of Clacification According to Segments					
	Maxillary			Mandibular		
	6 Years	8 Years	10 Years	6 Years	8 Years	10 Years
I_1	6.65	9.10	11.10	9.45	10.70	11.60
I_2	5.77	7.35	10.65	8.69	10.10	11.70
C	0.00	5.90	10.00	0.00	6.10	10.25
Pm_1	5.77	5.60	8.50	5.96	5.80	8.75
Pm_2	4.99	5.40	8.30	4.13	5.10	8.60
M_1	6.82	10.70	11.90	7.56	10.70	11.55
M_2	0.00	4.95	9.25	3.82	4.80	9.15

greater in the females than in the males. Sex differences were also present in the calcification of the permanent teeth. Mandibular central incisors showed the maximum root formation. The difference in the calcification of the maxillary and mandibular teeth was to the extent of 2 to 3½ stages, and the difference was particularly more in females than males. Mandibular central and lateral incisors were ahead in calcification to maxillary central and lateral incisors. The crown of second molar was not yet completed at the age of six years.

At the age of eight years, more than 7/8 of the root of central incisors were resorbed. The differences in the development between maxillary and mandibular teeth was maximum with respect to lateral incisors and minimum with the first molars. Between six and eight years calcification occurred more in maxillary central incisors, first molars and mandibular first molars. Crown of the second molar had been completed and the spicules had arisen from the mesial and distal end of the crown.

At the age of ten years, the root of the deciduous central and lateral incisors had been completely resorbed in both the jaws. The maximum resorption of deciduous teeth had taken place between six and eight years and there was relatively less resorption in the first and second molars between the ages of eight and ten years. During this period, the maxillary deciduous first molars showed practically no progress in the resorption of roots. As far as the development of permanent teeth are concerned at this age, it was found that more than 7/8 of the calcification was completed in the central incisors and first molars of both the jaws. Maxillary first molars were fully calcified. With the exception of the maxillary first and second molars calcification was usually more advanced in mandible than in maxilla. Calcification occurred more between the ages of eight and ten years in all the teeth except central and lateral incisors.

Comparison of the Findings with those of other Workers

In India no similar study has been done in the past and hence there have been no standards for the purpose of comparison. Data are, however, available on the European and American children. Their standards of growth and developments cannot be applied to the Indian Children, since it is believed that the growth of children in warmer climates is more advanced than that of children in temperate countries. In spite of the climatic and racial differences, a comparison of the present results with those available on European and American children, showed only minimal differences between the groups. More data covering the other age groups as well, will have to be obtained before any conclusions could be drawn.

110. Enquiry into the effect of sodium-hexametaphosphate in the treatment of periodontal disease under Dr. K. L. Shourie at the Sir C.E.M. Dental College, Bombay.

About 100 patients (Female) from the Shradhanand Mahilashram were examined for the presence of gingival disease with a probe and mirror. The disease was scored according to Russel's periodontal score. The D.M.F. score was also noted down. Patients were divided into two batches. Attempt was made to see that the average age and the periodontal score in the two batches were the same. One side (left) of the maxillary and mandibular teeth were scaled and polished. One batch was given sodium hexa meta phosphate solution and another ordinary water. The batches are being examined periodically.

111. Study of blood changes associated with periodontal disease under Dr. T. N. Chawla at the Dental College and Hospital, Lucknow.

Hæmatological constants, plasma iron and copper, serum proteins calcium phosphorus and alkaline phosphatase and blood sugar were estimated on a sample of 75 normal subjects, and the values compared with those obtained on 110 patients suffering from varying degree of periodontal disease.

The results showed that all other constituents except plasma iron and plasma copper are not significantly different in the two groups when analysed in terms of their average values. Patients with periodontal disease exhibited a hypoferræmia and hypercupræmia, the average values for plasma iron and copper being 88 and 162 per 100 c.c., respectively. These changes do not appear to be related to the severity of the destructive process. Only 12 out of 110 cases of periodontal disease showed anæmia which was moderate in its severity and normocytic in its morphology. Anæmia was unrelated to the degree of hypoferræmia or the severity of the disease. Some patients showed a rise in the total serum proteins which appeared to have been contributed solely by a rise in the globulin.

112. Investigation to gauge the value of various endodontal treatment procedures for the abscessed pulpless deciduous and young permanent teeth under Dr Vimla Sud at the Safdarjang Hospital, New Delhi.

So far 4,638 school children were examined, out of which 767 were found to be suffering from pulpless abscessed teeth. The number of teeth abscessed in them were 1,320. Of these, 67 have been given the endodontal treatment as follows :—

Nynol :	23
Zinc Oxide : Eugenol.	24
Ammoniacal Silver Nitrate :	20

These cases are being followed up at monthly and bimonthly intervals and their results are being gauged both clinically and radiographically. The results obtained so far are very satisfactory. Further work is in progress.

113. Analysis of foodstuffs for fluorine content at the King Institute, Guindy, Madras

In the first instance, the fluorine contant of different varieties of "Haldi" (Turmeric) was etimated. The estimation was done on dried samples by the distillation technique and the Scott Alizarin and Zirconium method. Twenty-nine samples of Turmeric from different part of India were tested. All but two of the samples showed a fluorine content btween 20 and 60 parts per million. One sample from Betul (Madhya Pradesh) showed the higest fluorine content (96 parts per million). It is considered worthwhile to examine more samples from this area and also determine the fluorine content of irrigation and ground water of this region.

114. Enquiry entitled "Prevalence of periodontal disease under Dr." T. N. Chawla at the Dental College and Hospital, Lucknow.

Work on the problem was started on 2nd June, 1958. To date 300 individuals have been examined by Russel's method and 450 by Ramfjord's method. Subjects examined by Ramfjora's method include both hospital patients who attended the dental out-patient as well as school children examined at their institutions.

The subjects were between the ages of 12 years and 30 years. Those who had prophylaxis during the six months prior to examination were excluded. A clinical chart for recording the data was prepared on lines similar to those recommended by Russell and Ramfjord. The detection of bacterial plaque was done by application of Bismark Brown solution.

Russell's Method

Only positive clinical signs were taken as an indication of disease. No x-rays were taken.

Using strong artificial light the gingival condition, oral hygiene, calculus and attrition were noted and scores for each, given on the basis of Russell's technic.

The method for oral hygiene and calculus was essentially similar to that given by Greene. Mouth was divided into six segments, three in each arch. The three segments were made up of right and left posterior teeth and the anterior teeth the dividing line being the distal of the cuspids.

Ramfjord's Method

The patient was examined under strong artificial light, using a mouth mirror, periodontal probe marked in milimeters, tweezers and Bismark Brown disclosing solution. The area surrounding teeth 3, 9, 12, 19, 25 and 28 that is $\frac{6/14}{41/6}$ were examined.

Gingival condition, calculus, attrition, mobility, drifting, pocket depth and plaque were looked for and scores given for each, and calculated according to the method of Ramfjord. The work is in progress.

115. An evaluation of traumatogenic occlusion as an etiological factor in periodontal disturbances under Dr. K. P. Choudhury at the K. G. Medical College, Lucknow.

The object of the study is to evaluate the nature of traumatogenic stresses and their relationship to the periodontal disturbances. To start with 100 cases have been examined for standardization of techniques, and subsequently 30 cases have been studied in greater detail.

The detailed investigation of each case included :—

1. History and clinical dental examination ;
2. Laboratory investigations on adjustable "HANAU" articulator;
3. Full mouth X-rays.

1. The examination is conducted for the state of dentition, and the standard of oral hygiene and the number of missing teeth, degree of calculus and attrition were noted. The condition of the gums was noted under the headings of colour, marginal contour, gum recession, papillary contour, texture, exudate and pocket depth. Mobility of teeth was noted by finger impact method. Casts were made from alginate impressions of upper and lower arches.

2. Models were mounted on articulators with the help of face bow and check bites. Premature contacts were noted by performing various movements of articulated models.

3. Full mouth x-rays were taken and examined for changes in periodontium. The work is progressing.

116. Enquiry on periodontal disease in Punjab under Dr. B. R. Vacher at the Government Dental College and Hospital, Amritsar.

The work on the enquiry was started on the 12th August, 1958. Over 600 cases were examined, (500 cases by Russell's Method and over 100 cases by Ramford Technique). Majority of these cases were the patients visiting the Dental Hospital, while the rest were the students of the Dental College, Amritsar. Arrangements are being made to conduct the examination of School Children and College students between the ages of 12 to 20 years.

Clinical examination of cases was conducted under artificial light and the instruments used were mouth mirrors, periodontal explorer and periodontal probe, scalers and tweezers, etc. Besides, solution of Bismark Brown was used for disclosing the bacterial plaque according to the Ramford Technique.

Ramford's as well as Russell's methods of examination and charting were employed. Green's method of determining and charting the oral hygiene and calculus score was included in the Russell's Chart. Ramford's method of examination and charting is rather time consuming, hence this chart has been modified to make the charting procedure simple.

117. Public Health Engineering Research unit under Dr. T. R. Bhaskaran at the All-India Institute of Hygiene and Public Health, Calcutta.

The Scientific Advisory Board of the Indian Council of Medical Research at its meeting held in 1956 recommended that a Public Health Engineering Research Unit be established under a Co-ordinating Officer who will organise, co-ordinate and stimulate research in the field of Environmental Sanitation in the country. The Unit started functioning at the All-India Institute of Hygiene and Public Health, Calcutta, from September, 1957.

During the year 1957-58 the Unit has carried out investigations on :—

(a) Industrial waste disposal and water pollution in U.P., Bihar and West Bengal States.

(b) Survival of tubercle bacilli in sewage treatment processes used in T.B. Sanatoria.

(c) Maintenance of tubewells in the rural areas of Bihar under Shri K. N. Rohatgi, Superintending Engineer, P.H. Engineering Dept., Patna.

In addition to organising work under the foregoing projects, the Co-ordinating Officer participated in several conferences, held in different parts of the country, on various aspects of public health engineering problems. He also rendered advice to the Damodar Valley Corporation and Sindri Fertilizers regarding the establishment of proper organizations for research on industrial waste disposal and water pollution problems, etc. He also visited the Bengal Paper Mills along with the Executive Engineer of Public Health Engineering Department of West Bengal to advise them on the disposal of paper factory effluent with special reference to pollution of the Damodar River.

At the request of the N.E.F.A. Government, the Co-ordinating Officer visited this region to study the problems of environmental sanitation and to suggest measures for improvement. A report on the existing conditions and suggestions for future development of environmental sanitation activities in this region has been submitted to the NEFA health authorities.

It is hoped that as a result of the work undertaken in U. P., Bihar and West Bengal, it would be possible to establish a proper organization and laboratory set-up for carrying out work on industrial wastes and water pollution and to evolve proper administrative procedure for mitigating the insanitary conditions arising out of improper disposal of wastes within the States.

118. Study on the different types of latrines used in rural areas under Prof. N. Majumdar at the All-India Institute of Hygiene and Public Health, Calcutta.

It is well recognised today that indiscriminate defecation in rural areas has been greatly responsible for the high incidence of gastro-intestinal diseases and worm infestation. In spite of concentrated efforts from the health departments of the different states, it has not been possible to get the latrine accepted by the rural people in the country. Absence of a satisfactory design of a suitable latrine for use in rural areas seems to be one of the major factors contributing to the non-use of the latrines in villages. Being aware of this important deficiency ICMR initiated a study with the object of evolving satisfactory latrines for these regions. Since septic tanks have been used in non-sewered areas frequently for the treatment of household sewage, a study was undertaken to examine the different designs followed in the country with a view to determine the most suitable one.

Four field units were sanctioned by ICMR in 1957, one each in the states of West Bengal, Kerala, Punjab and Bombay. Of these, the unit in West Bengal started functioning from the very beginning. The units in Kerala and Punjab started operating from 22.2.1957 and 20.8.1957, respectively. Permission of Government of Bombay was received for the establishment of the unit only in July 1958. The staff for this unit are now being recruited. Work is still to commence at this centre.

The unit in West Bengal, having examined some of the popular designs of water seal squatting plates used in the country, has developed a design a pan and trap which permits easy flushing with less than a half of a gallon of water. It has now been possible to cast this pan in one piece. The casting method has been improved and the finish obtained is fairly good. It has been possible to get this pan cast in the villages with the help of local labourers. The cost of this pan inclusive of depreciation charges is Rs. 1.60. Breakages in the casting have been minimized to an insignificant number. The pan is fairly light—10 lbs. in weight. A few of these pans have been installed in latrines in rural homes of West Bengal. These latrines have been under observation for the last 3 months. So far no complaints have been received.

The performance of 3 experimental septic tanks and one aqua privy was under observation during this period. The efficiency of the septic tankswith respect to B.O.D. removal is of the order of 92 pereent. A good direct correlation was observed between logarithin of raw sewage B.O.D. and logarithm of percentage removal of B.O.D. in two of the septic tanks. An equation for the line of regression was obtained and the constants for the equation determined. These equations for septic tanks Nos.I and II, are $E=60R^{0.056}$ and $E=56R^{0.0623}$, respectively, where E is the per cent removel of B.O.D. by the tanks and R is the B.O.D. of raw sewage in ppm,

Since the latrines were hand-flushed, the quantity of sewage per capita was small. Observations at three of these installations indicated per capita sewage of 1.3 gallons per day. Figures on per capta contribution of solids varied greatly in the three cases. Observations are still in progress in this respect. In spite of high percentage removel of B.O.D. in

the septic tanks, the effluent still has 150-300 ppm of B.O.D. and hence was not fit to be discharged in open drains. The effluent from these tanks was disposed of by open jointed tile gallery in two instances and in soak pits in the other two cases. The soil is a mixture of sand and clay. These disposal facilities have been in operation for more than 2 years in two cases and about a year in the other two instances and so far no complaints have been received. Sludge from two septic tanks was withdrawn under hydrostatic head. Laboratory examinations of the digested sludge indicated solid content in sludge of the order of 7 per cent and 11 per cent respectively.

The unit in Punjab observed the performance of a few test latrines during the current year. Different methods had been used for flushing these latrines. The serious objections to some of the types used, happened to be splashing of water during its use and large quantity of water required for flushing them. Observations there indicated that satisfactory flushing of these units might be possible with water from a funnel or a cistern placed at a height of 3' to 4' above the level of the pan.

The unit in Kerala has been interested in developing a cheap and suitable latrine for use in the water-logged areas. A separate note is submitted by the Chief Engineer of the state.

119. Enquiry to study the effect of temperature and time of storage on bacterial densities of water and to compare the British Ministry of Health and American Public Health Association techniques for quantitative detection of Coliform Group of Bacteria under Dr. S. V. Ganapati, Chief Water Analyst, Delhi, Joint Water & Sewage Board, New Delhi.

1. Introduction

There is considerable disagreement both on the maximum permissible time interval between sampling and bacteriological examination and on the method of storing the sample during this period. The Bacteriological Examination of Water Supplies (Ministry of Health, Report No. 71, 1939) recommends that samples should be despatched to the laboratory by the quickest route immediately after collection, the time occupied in transit being preferably less than 6 hrs. If a longer period is likely to elapse, the sample should always be packed in ice. In the "Standard Methods for the Examination of Water, Sewage and Industrial Wastes" (American Public Health Association and American Water Works Association, 1946) it is recommended that the period between sampling and examination should not exceed 6 hrs. for impure waters, and 12 hrs. for relatively pure waters; and that during transit the temperature should be kept between 6 and 10°C. The P.H.L.S. Water Application Form London, 1951, 1952, 1953 (a), 1953 (b), 1953 (c), & 1953 (d), states that samples should reach the Laboratory within 6 hrs. of collection.

Again, in the latest work on International Standards for Drinking Water published by the World Health Organization in September 1958 (p. 18, para 2.2.4) it is stated that "during the period elapsing between collection and examination the temperature of the sample should be maintained as closely as possible to that of the source of the sample at the time of sampling ; and when local conditions necessitate delay in excess of 24 hrs., consideration should be given to provide for a field examination of samples by making use of the Micro-filter technique or of temporary laboratory facilities at the site."

In India which is a land of distances and of extreme variations of temperature, delays in excess of 24 hrs. to 48 hrs. or even 72 hrs., are involved for transporting samples to a Central Public Health Laboratory for examination. The variations in bacterial densities of water samples which may take place under such circumstances are not known. Hence it is very necessary to find out authoritatively whether such delays are permissible even if the samples are refrigated or whether they are likely to vitiate the results considerably. An attempt is therefore made to find out a practicable standarised procedure for transportation and storage of samples between collection and subsequent examination so that the sanitary evaluation of water samples may be fully reliable.

2. The Technique employed in the Investigation.

The standard method for determining the number of coliform organisms in a sample of water described in the Ministry of Health Report (1939) is subject to a large error, both because of the small number of

tubes examined at each dilution, and because of the large dilution factor. From this point of view the method which has been adopted by the P. H. L. S. Water sub-committee for an identical investigation in England is more suitable. In the present investigation this 70-tube technique using twofold diminishing volumes of the samples is followed. The number of samples tested for presumptive coliform organisms so far, is 156 for iced and uniced samples taken together. A difference of nine positive tubes or more between the sample examined as soon after collection as possible, and after different hours of storage, *Viz.*, 3, 6, 12, 24, 48 and 72 at room temperature and in ice is taken as indicative of a real change in bacterial density of the sample (P. H. L. S., 1953).

3. Results of Examination.

(a) Storage at Room Temperature (16°C. to 29°C.)

On storage there was a tendency for the number of organisms in the water to change. After 3 hours of storage 8.33 per cent or approximately 1 in 12 showed a significant increase and 16.66 per cent or approximately 2 in 12 a significant decrease; after 6 hours of storage 8.33 per cent or approximately 1 in 12 showed a significant increase and 8.33 per cent or approximately 1 in 12 showed a significant decrease ; after 12 hours of storage 8.33 per cent or approximately 1 in 12 showed a significant increase and 25 per cent or 3 in 12 showed a significant decrease ; after 24 hours of storage 16.66 per cent or approximately 2 in 12 showed a significant increase and 25 per cent or approximately 3 in 12 showed a significant decrease ; after 48 hours storage 8.33 per cent or approximately 1 in 12 showed a significant increase and 33.33 per cent or approximately 4 in 12 showed a significant decrease; after 72 hrs. storage 16.66 per cent or approximately 2 in 12 showed a significant increase and 58.33 per cent or 7 in 12 showed a significant decrease in the number of organisms.

(b) Storage in Ice (1°C. to 10°C.) :

After 3 hours of storage 1 in 12 showed either a significant increase or decrease ; after 6 hours of storage 2 in 12 showed a significant increase and 1 in 12 showed a significant decrease ; after 1. hours of storage 1 in 12 showed a significant increase and 2 in 12 showed a significant decrease; after 24 hours of storage increase and decrease were equal being 2 in 12, after 48 hours of storage 1 in 12 showed a significant increase and 4 in 12 a significant decrease ; after 72 hours of storage 2 in 12 showed a significant increase and 4 in 12 a significant decrease.

The results of the investigations done so far show that up to 48 hours of storage either at room temperature or in ice, approximately 4 in 12 samples of water will at least halve their content of presumptive coliform count and 1 in 12 will at least double it. After 72 hours of storage at room temperature approximately, 7 in 12 samples of water will at least halve their content and 2 in 12 will at least double it, while in ice, approximately 4 in 12 samples of water will halve their content and 2 in 12 will double it.

INDUSTRIAL HEALTH

120. Industrial Health Research Unit under Dr. M.N. Rao at the All-India Institute of Hygiene and Public Health, Calcutta.

A. Air Pollution In Calcutta And Environs Studied For The Third Year.

(i) *Dust fall :*

The monthly average dust fall in Calcutta has been found to lie between 35 and 86 tons per sq. mile for these three years.

(ii) *Air-borne contaminants :*

The average monthly distribution for the last three years show the following ranges of concentration :

Sulfur Dioxide	0·021—0·058	ppm.
Oxides of Nitrogen	0·037—·122	ppm.
Ammonia	0·168—0 299	ppm.
Aldehyde	0·04 —0·15	ppm.
Air-borne dust	0·070—0·600	mg/M^3 air

(iii) *Diurnal variation :*

The results show much higher pollution of air in winter than in summer and monsoon.

(iv) *Rural environment :*

The results show much lower concentration of the air contaminants in the rural areas as compared to Calcutta.

B. Industrial Health Physics

(i) *Physical aspects of heat regulation.*—The statistically designed experiment on the inter-relationships between (a) grade of exercise, (b) duration of exercise, (c) evaporative water loss, (d) rectal temperature and (e) sweat conductivity, partially reported last year, has been completed. The climatic conditions of the experiment were as follows :

	Air Temp. °F	Wet Bulb temp. °F	Globe Thermo-meter °F	Air Velocity ft/min.	Effective temp.
Mean	95·0	84·7	91·4	17	86·8
Range	93·2—99·5	84·4—85·7	90·4—93.5	10—38	86·2—87·3

It has been possible to derive an index combining sweating rate and conductivity, which may have a significant bearing in the selection of personnel for work in hot and humid environments.

(ii) *Temperature co-efficient of sweat conductivity.*—The variation of specific conductivity with temperature of a number of sweat samples collected from different human volunteers has been studied and a co-efficient defining this variation has been derived. This temperature co-efficient has the value 0·02111, which compares very well with that of urine, namely 0.02039 reported previously. With the help of this co-efficient it is possible to reduce all conductivity values either of urine or sweat at a standard temperature, whatever be the temperatures at which they are actually measured after collection from the body. This helps scientific comparisons of experiments under different conditions.

(iii) Further research on Radiation Physics as envisaged during last year was discontinued due to advice from higher authorities.

C. Industrial Psychology

(i) Study on fatigue in industry :

The preliminary investigation on the problem of fatigue in industry was carried out in an engineering industry and in a glass moulding shop near Calcutta. Six workers of the machine shop were observed in all the three shifts : morning, evening and night. The result shows that 22.2 per cent of the working period in a shift was lost due to various personal causes, mostly avoidable. It was also observed that the total time lost due to personal causes was higher in the night shift than in the morning or evening shifts. An artificial limitation of individual productivity was set up by the factory authority by fixing up a quota. Lack of motivation was the most important inhibiting factor affecting individual productivity. Wage incentive alone was found not sufficient to increase productivity. A study on four blowers and four helpers in a glass moulding shop shows that the average daily individual production trend follows the normal fatigue curve. Controlled experiments have been taken up to determine the maximum individual production capacity in an unit work spell, to the optimum rest periods to promote the workers' efficiency and reduce their mental strain as far as practicable.

(ii) Standardization of psychological tests :

In continuation of the preliminary work reported earlier on dexterity and steadiness tests for use in vocational selection programmes suitable to Indian personnel engaged in precision jobs, a battery of psychological tests including Tweezer Dexterity, Steadiness and Cox Tests was administered to 25 male and 39 female workers of the mounting section of a lamp manufacturing industry near Calcutta. The reliability and the validity of both Dexterity and Cox Tests were determined. Significant difference in scores in these two tests between skilled and unskilled workers was obtained.

D. Social Sciences

The Medico-Social Worker and the Health Visitor completed a thome environment survery of 376 industrial workers attached to the Industrial Health Clinic of the Urban Health Centre Chetla. This is a part of a health survey including a study of the work environment and medical status of the workers preliminary to planning a co-operative medical care programme for the unregistered factories.

E. Applied Physiology

Assessment of physiological stress in hot industries :

Three groups of foundries, have been selected according to their existing environmental conditions. Investigation on one of these groups is in progress. Due to lack of field staff, work on the other groups could not be started. A comprehensive observation of environmental factors (*Viz.* dry bulb, wet bulb, humidity, effective temperature, etc.)and physiological responses (*Viz.* energy expenditure, water loss, circulatory stress, etc.) of the workers are being analysed.

Observations in the hot dry season are completed in one of the factories in the first group and investigations on two factories in the same group are in progress for hot humid season.

F. Industrial Medicine

The post of the Medical Officer of the Unit has been lying vacant during the period under review.

G. Services To Industry

The Unit as usual received a number of requests from various industries for technical advice and service. The following are the more important ones.

(i) An environmental health survey was done at the Tata Iron and Steel Factory at Jamshedpur, with refercnce to some air contaminant. like So_2, NH_3, dust, thermal stress, etc. A report on the findings and corrective measures to be adopted was given to the authorities concerneds

(ii) At the instance of the West Bengal Inspectorate of Factories, a survey in a representative group of pigment and paint factories was made for lead hazard.

(*iii*) *Head load study.*—The head load in a Government Instrument Manufacturing Factory has been investigated. Measures for the amelioration of the excessive thermal stresses have been recommended.

H. Workshop Activities

The workshop of the Industrial Health Research Unit continues to be as popular as ever, as evidenced by the number and variety of the jobs demanded of it which have been successfully completed during the year. A notable work done by the workshop unit this year is the design and construction of the I.C.M.R. exhibit on Industrial Health, now on view at the International Exhibition at Delhi.

121. Enquiry into the causes of absenteeism in an Industrial concern under Dr. H. P. Dastur at the Tata Industries Ltd., Bombay.

The main work was analysis of the whole data gathered on absenteeism of workers in the Carding and Spinning Departments. The procedure adopted for the classification of the data was the same for the two departments, i.e. workers were divided into groups of 25 each according to their serial ticket numbers. They were further classified according to age-groups, communities, occupations, wage-groups and tenure of service.

Classification for age-groups :—

1. 20 — 29
2. 30 — 39
3. 40 — 49
5. 50 and above.

Classification for communities :—

1. Maratha.
2. Bhaiya.
3. Minorities.

All the different sub-occupations in both the Departments were taken for classification.

Wage-group classification :—

	Rs.		Rs.
1.	90	—	120
2.	121	—	150
3.	Above 150.		

Tenure of service classification :—

1. 0 to 2 years
2. 2+to 5 years
3. 5+to 10 years
4. Above 10 years.

Data were analysed according to the following factors :—

1. Rate of general absenteeism.
2. Causes of absenteeism and man-days lost under each cause,
3. Frequency of absence and its nature.
4. Absence in relation to age, community, occupation, wage-group and tenure of service.

Proportions of each cause to total unauthorized absence and absences for *'D', 'E', 'J' and 'O' causes were calculated for wage-groups.

* D=Indebtedness ; E=Worker's discontent with work, co-workers or supervisors ; J=Drink addiction and O=Miscellaneous.

122. Enquiry in Bagassosis at the Vallabhbhai Patel Chest Institute, Delhi.

The co-operative research programme on different aspects of Bagassosis was continued during the year under review :

I. Clinical

A clinical survey of the incidence and nature of ill-health produced by inhalation of bagasse dust among factory workers at Dalmianagar was undertaken. Certain difficulties including considerable resistance from the workers have been experienced in the initial stages of survey. In spite of various obstacles, a total of 253 has been examined in 1958 alone. Arrangements have been made to replace the existing temporary labour with more stable labour hired directly by the factory in order to facilitate continued observation on new recruits for a reasonable length of time.

Apart from taking detailed history, a complete physical examination including radiography of the chest, examination of the blood, urine and stool, exercise tolerance and pulmonary function tests and skin test with bagasse antigen were carried out.

It was found that 80 per cent of the old workers had symptoms of cough and breathlessness on exertion. Majority of them had auscultatory findings in the lungs. Some had low grade fever. Radiological signs consisted of increased hilar shadows and increased broncho-vascular markings. In a few cases there was a gradual aggravation of radiological signs. In some there was definite deterioration in ventilatory efficiency as revealed by the pulmonary function tests.

Published reports so far have mentioned only of acute respiratory disorder with symptoms like cough, breathlessness and fever. The present survey indicates that a more chronic type of mild respiratory disability which does not necessarily make the workers report sick, is possible. Further work is required to determine the nature and character of this disability from the clinical point of view.

II. Pathology

Experimental studies have been extended to other animals like monkeys in addition to guinea pigs and rabbits in the light of the earlier observation that the type of cellular reaction differs in different species. Moderate differences have been observed which distinguish the changes in monkey lung from those observed in guinea pigs. Differences have been seen in the basic cellular reactions in animals treated by inhalation, intratracheal insufflation and injection with sterilized and unsterilized bagasse. One animal injected I. V. with suspensions of crude bagasse showed large areas of necrosis in many organs, caused by an actively growing fungus. The changes in the lung induced by exposure to the dust in a chamber or in the factory over longer periods of time have been studied. These studies indicate that the changes are diffuse and bronchopneumonic in nature, could be initiated as early as two hours and that they tend to aggravate with time, resulting in fibrosis. They

however, need confirmation. The changes are also reversible up to a fairly late stage of exposure. Studies on rabbits receiving repeated injections of bagasse have shown that the animals become hypersensitive to some factor in the bagasse at some stage of the exposure. This may act as one of the aggravating factors in the later stages. The effect of *Aspergillus niger*, a fungus chiefly incriminated in the past as the cause of this disease has been studied.

III. Biochemical

As the tissue respiration of lung from bagasse-exposed animals gave a higher respiratory quotient as compared to normals, important respiratory enzymes like succinic dehydrogenase, cytochrome oxidase, adenosine triphosphatase, malic dehydrogenase, etc., were studied in the and insert lungs of guinea pigs exposed to bagasse. Blood samples of the workers exposed to bagasse dust at the Dalmianagar factory were examined for changes in the pattern of blood constituents like cholesterol, chloride, silica, sodium, potassium and serum proteins.

IV. Mycological and Bacterial

The fungi isolated from samples of bagasse were identified and classified into 12 gen and insert era and 18 species. The study indicated that *Helmonthosporium sp*, *Collectotrichum falactum* and *Glaveosporium sp* were characteristically associated with bagasse. Another commonly associated species, isolated from bagasse was pencillium. Sputa collected from the workers at the factory were also cultured. Some important fungi isolated include *Candida albicans*, *Aspergillus niger* and an undetermined species of *Candida*. Some ubiquitous saprophytes like Mucor and Rhizopus were also occasionally isolated. One significant isolation was that of a species of pencillium in pure culture from one specimen. A Friedlanders bacillus like organism was found in 10 per cent of the sputa collected in Dalmianagar. In another batch of material cultured in this Institute 10 out of 13 sputa, showed the same organism. Lungs obtained from animals inoculated with a bagasse suspension of determined particle size were cultured forsolation of fungi. A species of pencillium was isolated from the lungs of one of the animals.

MATERNAL AND CHILD HEALTH

123. Studies in Rh sensitization under Dr. V.S. Mangalik at the K.G. Medical College, Lucknow.

Determination of Rh Factor was carried out both in men and women. The tested population was divisible into four groups:—

(a) Females who had not been pregnant before.

(b) Expectant mothers.

(c) Females who had been pregnant before.

(d) Males.

In all 3,606 persons were tested. Of these 2,335 were expectant women. Among these females, in 550 it was possible to investigate both the mother and the new born baby.

ABO and Rh(D) blood groups were determined in all investigated cases. D-negative samples were further studied for the detection of Du antigen by the use of anti-human globulin serum. All sera obtained from Rh negative mothers, whether primipara or multipara, and whether they gave a history of repeated abortions or not, were examined for the presence of antibodies against D both in saline and protein menstrum. Attempts were made to contact the husband and children of these Rh-negative mothers for the determination of Rh (D) and ABO blood group but this did not meet with any success.

Almost all the immune sera employed by us were manufactured by Ortho-Pharmaceutical Corporation, New Jersy, U.S.A. They were of uniform high titre and avidity. At later stages those supplied by Tata Memorial Institute of Cancer Research were employed.

Samples of blood were obtained from the vein, and all tests were carried out on clotted blood. In the new born, the cord blood was tested.

RESULTS.

1. Total Populations.

The incidence of Rh blood grous (D) in 3,606 persons examined is shown in Table I, along with the distribution of ABO blood groups in the Rh-positive and Rh-negative groups. The frequency distribution of both Rh (D) and ABO groups in the whole population and in different castes and communities is under analysis.

TABLE I.

Incidence of Rh Factor in the Mixed Population.

Total No. of cases 3606				DD or Dd 3554				dd 48 (1.6 per cent)			
'A'	'B'	'O'	'AB'	'A'	'B'	'O'	'AB'	'A'	'B'	'O'	'AB'
758	1411	1083	354	741	1395	1069	349	15	14	14	5
21.0 Per cent	39.1 per cent	30.1 per cent	9.8 per cent	20.8 per cent	38.9 per cent	30.5 per cent	9.8 per cent	31.4 per cent	29.1 per cent	29.1 per cent	10.0 per cent

2. *Expectant Women.*

In all 2,335 expectant women were examined. Out of these, it was possible to obtain samples of blood both from the mother and the new-born in 550 cases. The distribution of Rh in relation to other groups in these women is shown in Tables II, III & IV.

TABLE II.

Incidence of Rh Factor in Expectant Women.

Total Tested.	DD or Dd	dd
2335	2321	14 (0.6 per cent)

TABLE III.

Distribution of ABO Groups in Rh Negative Women

Total	Group 'A'	Group 'B'	Group 'AB'	Group 'O'
14	5	2	3	4
Percentage	35.6	14.4	21.4	28.6

TABLE IV.

Distribution of ABO Groups in Rh Positive Women.

Total	'A'	'B'	'AB'	'O'
2321	501	929	261	630
Percentage	21.5	40.0	11.0	27.5

A history of caste and community was obtained in 1,606 women. The distribution of both ABO and Rh (D) groups in these women is presented in Tables V & VI.

TABLE V

Community-wise Distribution of ABO Groups in 1606 Samples

Community	Total No.	A		B		O		AB	
		No.	percent	No.	percent	No.	per cent	No	per cent
Hindu	1,404	276	19.7	547	38.9	482	34.3	99	7.1
Muslim	164	39	23.8	68	41.4	41	25.0	16	9.8
S ikh	18	3	16.6	10	55.6	4	22.2	1	5.6
Christian	20	2	10.0	15	75	1	5	2	10

TABLE VI.

Community-wise Distribution of Rh Factor in 1,606 Samples.

Community	DD or Dd	dd	Total
Hindu	1,390	14	1,404
Muslim	164	0	164
Sikh	18	—	18
Christian	20	—	20
Total ..	1,592 (99,07 percent)	14 (0.93 percent)	1,606

As may be observed, the most common group in the investigated population was B, the others in order being O, A and AB. There was no significant difference between Hindus and Muslims. The number of Sikhs and Christians examined was too small.

Rh Negative Women: (14)

The incidence of (dd) was comparatively much less in women than in men. The respective figures in the two sexes were 0.6 and 1.6 per cent. Among Rh negative women, two cases were of abortion, and in the others 8, babies were born alive, and in good health and did not show any evidence of hæmolytic disease. In 6 of the mothers and their children direct and indirect Coomb's test were performed. Analysis of these cases is presented in Table VII.

TABLE VII

		Mothers		Babies			
Sr. No.	ABO Gr.	Direct Goomb's Test	Indirect Coomb's Test	Rh. Gr.	ABO Gr.	Direct Coomb's Test	Indirect Coomb's Test
1.	O	—	—	+	O	—	—
2.	O	NOT	DONE	—	A	NOT	DONE
3.	B	—	—	+	A	—	—
4.	A	—	—	Aborted			
5.	B	—	—	+	B	—	—
6.	AB	NOT	DONE	+	B	NOT	DONE
7.	O	—	—	+	B	—	—
8.	O	NOT	DONE	(N—O—T		D—O—N—E)	
9.	AB	—	—	+	B	—	—
10.	O	—	—	(A—B—O—R—T—E—D			

Abortions.

In the Rh negative group the percentage of women who gave history of abortions was significantly higher than these in the Rh positive group. Of 2335 Rh positive women, history of abortions was elicited in 45 per cent whereas in the Rh negative group as many as 85.7 per cent gave history of abortion.

New Born Babies.

In all 550 samples of cord blood was examined. Of these there were 9 instances of dd. The rest were Rh positive. Distribution of ABO groups in both Rh positive and Rh negative babies is shown in Tables VIII & IX.

TABLE VIII.

Distribution of ABO Groups in Rh Negative Babies.

Total	Group 'A'	Group 'B'	Group 'O'	Group 'AB'
9	1	6	2	0
Percentage	11.1	66.6	22.3	

TABLE IX.

Distribution of ABO Groups in Rh Positive Babies.

Total	Group 'A'	Group 'B'	Group 'O'	Group 'AB
541	74	225	16	226
Percentage	13.6	41.6	2.1	41.9

24. Study of Rh factor in pregnant mothers under Dr. Subodh Mitra and Dr. Santosh Mitra in collaboration with Dr. C. R. Gupta and Dr. R. K. Dutta Chaudhuri at the Chittaranjan Cancer Hospital, Calcutta.

The object of the Scheme

The purpose of this enquiry is to study the incidence of Rh-factor in pregnant mothers and to find out the relationship if any, to neonatal deaths, neonatal jaundice, still-births, abortions and toxæmias.

Materials and methods

The source of material was from the antenatal outdoor and indoor of Chittaranjan Seva Sadan College of Obstetrics Gynæocology and Child Health. The techniques recommended in the Schedule of I.C.M.R. were being followed.

Results

Samples from	*No.*
Expectant mothers	1303
Cord ..	1304
Husband ..	32
Total No. of Rh Tests/done ..	2639
Rh positive ..	2544 (96.4 per cent)
Rh negative ..	95 (3.6 per cent)

	Rh positive	*Rh negative*
Brahmin	389 (95.7 per cent)	16 (4.3 per cent)
Kayestha	474 (95.6 per cent)	22 (4.4 per cent)
Vaisya	102 (95.1 per cent)	5 (4 9 per cent)
Others	283 (95.5 per cent)	13 (4.5 per cent)
Total	1248 (95.8 per cent)	55 (4.2 per cent)

Total No. of expectant mothers tested 1303.

Blood grouping (ABO) in expectant mothers and its relation to castes.

	Group A	*Group B*	*Group AB*	*Group O*
Brahmin (405)	107 (26.5 per cent)	131 (32.3 per cent)	31 (7.6 per cent)	136 (33.6 per cent)
Kayestha (495)	132 (26.6 per cent)	148 (29.9 per cent)	37 (7.4 per cent)	178 (36.1 per cent)
Vaisya (107)	26 (24.3 per cent)	39 (36.5 per cent)	6 (5.6 per cent)	36 (33.6 per cent)
Others (296)	79 (26.8 per cent)	125 (42.3 per cent)	9 (3 per cent)	83 (27.9 per cent)
1303	344 (26.4 per cent)	443 (34 per cent)	83 (6.3 per cent)	433 (33.2 per cent)

Total No. of Coomb's tests/done.. 1014

Coomb's negative	..	1014
positive	..	Nil

Total number of Du done .. 17

Du negative	..	17
Du positive	..	Nil

Blood grouping (ABO) of Rh negative mother

Group A	..	14 (25.3 per cent)
B	..	18 (32.8 per cent)
AB	..	5 (9.1 per cent)
O	..	18 (32.8 per cent)
		55

Incidence of abortion in expectant mother (1303) and its relation to RH factor.

Total No. of abortions in expectant mothers 58–of which

Rh positive	..	55 (4.2 per cent)
Rh negative	..	3 (0.2 per cent)

Incidence of Still births in expectant mothers 1303 and its relation to Rh factor.

Total number of still births..78 (6 per cent) of which

Rh positive	..	72 (5.5 per cent)
Rh negative	..	6 (0.5 per cent)

Incidence of neo-natal deaths in expectant mothers (1303) and its relation to Rh factor.

Total number of neo-natal death 107 (8.2 per cent) of which

Rh positive	..	:5 (7.2 per cent)
Rh negative	..	12 (1 per cent)

Incidence of toxæmia in expectant mothers (1303) and its relation to Rh-factor.

Total number with Toxæmias–108 (8.2 per cent) of which

Rh positive	..	106 (8.2 per cent)
Rh negative	..	2 (0.1 per cent)

Observations

It is evident from the results stated above that, the incidence of Rh negative cases was 3.6 per cent when whole series was considered together, but it was 4.2 per cent when only the expectant mothers were considered. This figure is higher than 3.28 per cent which was the incidence of Rh negative women recorded last year. This might be due to the fact, that more cases (1303) were studied this year as opposed to 487 covered last year.

In spite of all efforts, only 19 husbands of Rh negative mothers were available. All of them were Rh positive, as also were their children.

There are a few interesting cases in this study which are worth mentioning here. It is always expected that Rh negative mothers with Rh positive husbands, giving typical histories of Rh incompatibility, e.g. neonatal deaths, neonatal jaundice, still births, abortions, etc., should ordinarily show the presence of Rh anti-bodies in their blood. The following are the histories of three cases, where mothers were Rh negative with their husbands Rh positive.

C.N.	No. of Pregnancies														Coomb's Test	Test for Antibodies
	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
687	L	SB	L	SB	L	NND	L	L	NNDJ						neg	neg
920	L	L	L	NNDJ	NNDJ	NNDJ	NNDJ	—	NNDJ	NNDJ	NNDJ	L			neg	neg
1850	L	L	L	L	NND	NND	NND	NND	NND	NND	NND	SB	SB	SB	neg	neg

C.N.—Case number
L—Living
SB—Stil ibirth
NND—Neonatal death
NNDJ—Neonatal death with Jaundice

No anti-bodies in mothers could be demonstrated in any of the above cases. From the obstetrical histories it appeared as though these were typical cases of Rh incompatibility but after a thorough investigation it was difficult to attribute them to Rh factor, as no anti-bodies could be demonstrated in the mother's blood even immediately after deliveries.

Regarding the relationship of caste to the incidence of Rh factor in the expectant mothers, no significant variation could be demonstrated.

In the total population of expectant women, the commonest blood group was Group 'B' but caste-wise Group 'O' was commoner amongst Brahmins and Kayesthas and Group 'B' amongst Vaisyas and other castes.

No definite correlation could be demonstrated between the Rh factor and the incidence of toxæmias, abortions still births and neonatal deaths and jaundice except that the incidence of still births and neonatal deaths was slightly higher, (1 per cent and 0.5 per cent as against 0.2 per cent and 0.2 per cent respectively observed last year) among the Rh negative mothers.

125. Enquiry on the incidence and causes of still births and neo-natal deaths under Dr. P. M. Naidu and Dr. V. Gopal Rao at the Osmania Medical College, Hyderabad-Deccan.

During the year under report 96 cases of still births and neonatal deaths were available for autopsy and detailed histopathological studies.

During this period there were 11,170 births, out of which 636 cases of fœtal loss were recorded giving the peri-natal mortality rate of 56.9 per 1,000 births, as against 79.6 per 1,000 reported earlier (1953—55). This appreciable reduction in peri-natal mortality, among other factors, may be attributed to improved obstetric care instituted as a result of experience gained in the previous years.

In the present series it has again been observed that the principal clinical causes leading to peri-natal death were trauma and stress of labour, toxæmia of pregnancy, ante-partum hæmorrhage and maternal diseases which accounted, respectively, for 39.26 ; 11.65 ; 6,74 and 5.52 per cent of fœtal and no-natal deaths. Fœtal diformity accounted for 9.81 per cent, infection including syphilis, 7.97 per cent. Hæmolytic disease of the new born due to Rh incompatibility led to two fœtal deaths in the 96 autopsied.

Out of the total of 163 still births or neonatal deaths that occurred during the year 28 or 17.7 per cent showed no adequate maternal obstetric or pediatric cause. Out of this number, 11 were available for autopsy and this revealed that in 5, death was due to fœtal anoxia although clinical examination alone had failed to reveal the condition. In the remaining 6 the fœtuses were macerated and the cause of death could not be ascertained even after autopsy.

Morbid anatomical studies on the above 96 cases revealed that in 30 (31.24 per cent) cases, the lesions were tissue changes attributable to anoxia,such as intense congestion, œdema, peticheal or large visceral hæmorrhages and fatty degeneration in various organs like heart, live and kidney. In 14 (14-58 per cent) cases death was due to pulmonaryr lesions such as massive aspiration of amniotic fluid aspiration pneumonia and bacterial infections, this also included three cases of "pulmonary hyaline-membrane" disease. In 13 cases the cause of death was intracracranial hæmorrhage ;congenital anomalies accounted for 9 deaths. and these were as follows :—

Congenital anamolies.	*No. of cases.*
1. Polycystic kidney (one unilateral and one bilateral).	2
2. Hydrocephaly ..	1
3. Under-developed lungs ..	1
4. Diaphragmatic hernia ..	2
5. Agenesis of adrenals ..	1
6. Atresia of the duodino Jejunal junction	1
7. Anencephaly ..	1

In the present series syphilis was seen in only 2,08 per cent of cases autopsied as against 8.1per cent reported in the earlier series. Erythroblastosis fœtalis accounted for two deaths in the present series, whereas in the previous series, none was observed lastly in 25 cases the fœtuses were macerated, hence no morbid anatomical lesion could be defined. However, out of this number 8 cases were associated with trauma and stress of labour, 6 toxæmia, 4 maternal diseases including anæmia and 1 antepartum hæmorrage.

The Health Visitor has been carrying out the survey of socio-economic conditions of affected mothers. During the year under report she has made 280 home visits of mothers of cases of still-births and neo-natal deaths and 95 ante-natal cases. From June 1958 she is also engaged in collecting data regarding consanguinity of the affected mother according to a standard proforma. The inquiry is still in the initial stages but it is hoped that the data when analysed would throw light on genetic factors, if any, on the incidence of peri-natal mortality.

126. Enquiry into the causes of pre-natal deaths under Dr. A. Bhomik and Dr. S. De at the Medical College, Calcutta.

The enquiry was started on 21.6.56 and the report of 232 post-mortem findings was submitted earlier. During the year under review it has been possible to make another 200 post-mortem examinations of which 87 were on stillborn fœtuses and 113 were on neonatal dead babies. The naked-eye and histological findings revealed the following causes of stillbirths and neonatal deaths.

ANALYSIS OF THE CAUSES OF DEATHS

Still-Births (Fresh).

Group A.	*Mature.* (45)		*Premature.* (42)	
	No.	per cent	No.	per cent
(i) Trauma.				
(a) Intracranial Hge.	25	55.5	9	21.3
(b) Other trauma	0	0	1	2.4
(ii) Intrauterine aspyxia	14	31.2	19	45.2
(iii) Pneumonia	0	0	4	9.5
(iv) Congenital abnormality	4	8.9	3	7.2
(v) Gross prematurity	0	0	3	7.2
(vi) Undetermined	2	4.4	3	7.2

Group B. *Neonatal Deaths.113*

	Mature. (29)		*Premature.* (84)	
	No.	per cent	No.	per cent
(i) Trauma:				
(a) Intracranial Hge.	5	17.2	10	11.9
(b) Intracranial stress	1	3.5	3	3.6
(ii) Asphyxia	5	17.2	14	16.6
(iii) Pneumonia	10	34·5	31	36.9
(iv) Hyaline membrane	3	10.3	6	7.2
(v) Atelectasis	1	3.5	5	5.9
(vi) Congenital abnormality	2	6.9	2	2.4
(vii) Others	2	6.9	10	11.9
(viii) Undetermined	0	0	3	3.6

As in our previous report, the incidence of pneumonia in the current series also remains high (22.5 per cent) and the incidence of hyaline membrane disease (8 per cent) low. The incidence of intracranial hæmorrhage in the current series has been 24.5 per cent which has been higher than our previous observation. The work is continuing.

127. Study on chlorpromazine in the field of obstetrics under Dr. S.C. Bose at the Medical College, Calcutta.

The object of this enquiry is to study the effect of chlorpromazine hydrochlor as an analgesic in labour and for control of toxaemia of pregnancy by clinical trials on selected cases as compared to similar cases kept as control.

1. As an Obstetric analgesic. The subjects included were divided into the following groups:—

Group 'a' receiving 25 mg. chlorpromazine (I.M.), when the labour is well established—128 cases.

Group 'b' receiving chlorpromazine (25 mg.), pethidine 100 mg. and Pitocin drip (2 units)—5 cases.

Group 'c'—same as group 'a' with injections of pethidine—100 mg. (I.M.)—136 cases.

Control group—receiving morphine, barbiturates, bromides, etc.—600 cases.

Injections of promethazine hydrochlor (pheargan) 50 mg. was given to 92,3 and 89 cases of groups (a), (b) and (c), respectively.

Chlorpromazine was repeated in 50 mg. (I.M.) dosage where the initial dose failed to produce satisfactory effects within two hours. The labour was closely watched for maternal and fœtal effects.

Inferences drawn were as follows :—

(i) Chlorpromazine alone can produce good analgesic effect where the pain is associated with fear complex.

(ii) Chlorpromazine with pethidine gives a better result when the pain is due to some spasmodic facts.

(iii) The course of labour is not altered much except in a few primigravidae.

(iv) The drug does not produce any harmful effects on mother or fœtus. On the other hand, it allays vomiting, restlessness, etc.

(v) The action of the drug is not uniform.

(vi) Chlorpromazine pethidine drip with pitocin is the most satisfactory method and should be studied more critically in future.

2. Chlorpromazine in toxaemia of pregnancy :—

The effect of thedrug is being studied in three groups of pre-eclamptic toxaemia cases tostudy if it is possible to control their Progress to eclampsia.

Group—I—receiving 50 mg. chlorpromazine with 50 mg. Promethazine three times a day—45 cases.

Group—II—same as Group I with injections of pethidine 100 mg. twice daily—54 cases.

Group—III—A control group treated with morphine, mag. sulph, barbiturates, etc.—19 cases.

The patients were kept under observation, the various changes noted.

Following inferences were drawn :—

(i) The drug is able to bring down the blood pressure and the effect is more pronounced in patients with very high blood pressure.

(ii) An increasing dosage of the drug is required for control of blood pressure as the treatment proceeds.

(iii) The fall of blood pressure is transient.

(iv) Water excretion, changes in weight gain and control of œdema etc. do not differ significantly from control group.

(v) The effect of the drug is not uniform in all cases and it often produces a weakening sensation.

(vi) No hepatotoxic or allergic manifestations were found in the cases having a prolonged course of treatment with the drug.

128. Clinical and hormonal studies in women with primary and secondary amenorrhœa under Dr. L. V. Phatak at the G. R. Medical College, Gwalior.

Eighty five cases with a complaint of amenorrhœa were investigated from June 1957 to September 1958. The criteria for selecting a case were :—

1. Primary amenorrhœa in patients aged 18 years or more.

2. Secondary amenorrhœa in patients up to 35 years of age with the duration of amenorrhœa of 3 months or more.

3. Lactational amenorrhœa only when it persisted after weaning the child for 3 months or more.

A detailed and careful history of the patients with special reference to the tubercular infection, infectious diseases, mental disorder, etc., likely to be contributory to the development of amenorrhœa were obtained. Family history with reference to the occurrence of amenorrhœa in the mother or sisters was also elicited. Possible sources of tuberculous infection in the family were carefully looked for and an inquiry into the menstrual history and the age of menarche, etc., was made. In the obstetric history, the occurrence of complications, if any, in the antenatal period or puerperium was noted. A complete systemic and gynaecological examination was carried out.

As a rountine, complete blood picture with E.S.R., W.R. or Kahn test, urine examination, vaginal cytology and endometrial biopsy were done in all cases. In some cases additional investigations, e.g. hysterosalpingography, x-ray of the chest and B.M.R. were done, when felt necessary.

Observations

Out of 85 cases that were studied, 8 were of primary amenorrhœa and 77 cases were of secondary amenorrhœa. Most of the patients belonged to the poor economic group i.e. with income of less than Rs. 100 per month. The age of primary amenorrhœa cases ranged between 18 and 25 years, the average being 21 years, and in the secondary amenorrhœa the range was from 16 to 35 years, the average being 23·8 years. The duration of secondary amenorrhœa was from 3 months to 10 years, in 46 cases it was less than a year. Out of 77 cases suffering from secondary amenorrhœa 45 were nulliparous and 32 belonged to the parous group.

The height and weight ratio was proportionate in 67 out of 85 cases.

Sixtyfive cases out of 85, had suffered from one or more infectious fevers in the past prior to amenorrhœa. Four cases gave history of having had an emotional shock before the amenorrhœa developed. In cases a history of post-partum haemorrhage could be elicited in the previous delivery. In 7 cases a history of puerperal sepsis was obtained

The most common associated symptoms were backache and abdominal pain. Fourty four cases had backache out of which 35 had some associated pelvic lesion (tubo-ovarian masses, retroversion etc.,) 31 cases complained of abdominal pain and of these 16 had inflammed adenexal masses and a fibromyoma was present in one. Twenty six cases had a history of dysmenorrhœa. Vaginal discharge was complained by 30 cases which could not be explained very satisfactorily.

In the laboratory investigations, urine and blood examinations were normal in almost all cases. Only ten cases had a W.B.C. count of above 10,000 cells per cu.mm. of blood and E.S.R. was above 30 mm. per hour in 24 cases. W.R. was negative in all the cases.

Endometrial biopsy could be done in 61 cases. Material was too scanty for a report in 18 of these, while only necrotic material was obtained in 3. In 11 cases a definite tuberculors lesion, in 1 sarcoidosis and in 1 presence of foreign body gaient cells were reported. In the remaining 27 cases various types of endometrial patterns were present.

In 5 out of 24 cases in whom the endometrial biopsy could not be done, the uterus was absent, and in 4 cases the sound could not be passed. The remaining 15 cases failed to report or if reported were not in a fit condition for biopsy.

Vaginal cytology revealed good estrogen activity in 52 cases, while 30 patients showed hypo-estrogen response.

Associated lesions of the adenexa were present in 23 cases. 5 cases had bilateral and 14 had unilateral tuboovarian masses, while the remaining 4 had one sided thickening of the tube only. Of the 23 cases with some degree of adenexal inflammation, 10 had endometrium positive for or suggestive of tuberculosis, 6 had associated abdominal tuberculosis and 3 were associated with tuberculosis of the lungs. Thus 19 out of these 23 cases showed evidence of tubercular infection.

Of these 85 cases a definite cause could be detected in 50 cases, i.e. 58·8 per cent which is a relatively high figure compared to certain reports in literature.

The study also showed that in a considerable number (i.e., 32 out of 85 cases) tubercular infection is directly or indirectly responsible for amenorrhœa in these women.

129. Enquiry on hormone assays for Pregnanediol, oestrogens, chorionic gonadotropins and 17-ketosteroids under Dr. S. Mitra at the Chittaranjan Cancer Hospital, Calcutta.

In the year under review, the excretion rates of pregnanediol, œstrogens and 17-ketosteroids have been studied in individual cases week by week during pregnancy and after confinement from 121 samples (5 follow-up cases) of urine in normal pregnancy and 72 samples (3 follow up cases) of urine in toxaemia of pregnancy. The cases studied are divided into three groups :

(I) Normal pregnancy with term delivery.

(II) Normal pregnancy with premature delivery.

(III) Toxæmia of pregnancy (two premature delivery and one full term delivery.

Throughout the investigation, constant efforts have been made to ensure full collection of 24 hours urine.

Pregnanediol

The average excretion of pregnanediol in the present series varied from 9.65 mg./24 hours (18 weeks) to 45.36 mg. 25 hours (38 weeks) in normal pregnancy at term delivery (Group I). The results are in good agreement with those of other workers (Wilson & Randell, 1939 ; Cope, 1940 ; Watteville, 1951 ; Coyle 1956). The excretion pattern of pregnanediol shows a steady rise with advancement of pregnancy and a fall in the excretion 2 weeks prior to labour. The behaviour of pregnanediol in the cases delivered prematurely (Group II) is almost the same as at term delivery of Group I and the "peak excretion" (37.02 mg. 24 hours) is observed in the 34th week. The delivery took place on the 36th week Lyon (1941), Marrian (1949) and Mauzey (1950) noticed the same drop of pregnanediol excretion level one to two weeks before delivery.

In the urine of 1st, 4th and 7th day of post-confinement period the excretion of pregnanediol has been found to have a higher level (4.69 mg./24 hours) on the 1st day gradully declining to the value of 0.67 mg./24 hours on the 7th day after confinement.

In toxæmia of pregnancy at term delivery (Group III) (case VIII) the excretion was 19.64 mg./24 hours (31 weeks), as against 29.93mg/24 hours in normal pregnancy at the same week. This reduction in intoxæmia is significant. Other cases of toxæmia of pregnancy also show a lowlevel though the pattern of curve corresponds to that of a normal pregnancy curve.

17-ketosteroids

The average daily output of 17-ketosteroids estimated by carrying out the Zimmerman's reaction has been found to vary from 5.83 mg. (18 weeks) to I4.82 mg. (40 weeks) per 24 hours in normal pregnancy and from 4.23 mg. (18 weeks) to 14.6 mg. (40 weeks) per 24 hours in toxæmia of pregnancy. Unlike previous year's study, the excretion of 17-ketosteroids showed a slight rise with the progress of pregnancy. The rise may be attributed to the hyperfunction of adrenal cortex during pregnancy.

The excretion of 17-ketosteroids in toxæmia of pregnancy does not exhibit any marked change though it has been assumed that there should be an increase in its excretion in this condition.

Oestrogens

Urinary œstrogen estimation is beset with many difficulties. The loss of œstrogens occur during hydrolysis, extraction, partition or fractionation and even in the process of colour development. Various measures have been taken to minimize the loss (Bauld, Brown, 1954). But from the recovery experiment it has been shown that all the steps are not completely free from errors. Partition chromatographic method of Braunsberg (1954) is considered relatively more accurate and easier for the purpose of routine work and has been followed in this laboratory since 1956. Another recent method of Brown (1955) has also been developed successfully in this laboratory,

The average excretion of œstrogens in normal pregnancy at term delivery (Group I) ranged from 4.95 mg. (18 weeks) to 22.87 mg. (40 weeks) per 24 hours. Oestrogens showed a steady rise reaching the peak excretion in the last week of gestation. In the premature delivery cases (Group II) the excretion of œstrogens also shows the gradual rise from 4.1 mg. (18 weeks) to 17.1 mg. in the 34th week and then declined to 13.1 mg. in the last week of gestation. While in the Group I (delivery at term) the fall was observed from the first day after confinement only.

In toxæmia of pregnancy the excretion of œstrogens has been found to be definitely lower than that in normal pregnancy. At 31 weeks (Case VIII, Group I the total œstrogen is 6.62 mg. per 24 hours while the same in normal pregnancy (Group I) is 13.35 mg. per 24 hour. The other toxaemic cases also showed a law level during the manifestation of toxaemic symptoms.

A sharp fall in the urinary level of œstrogens in post-confinement period has been noticed. The average excretion varies from 4.213 mg. (1st day after confinement) to 0.230 mg. (7th day after confinement) per 24 hours, thus attaining nearly the pregnant level within a week.

From a study of five normal cases and three toxaemic cases it is realized that the work should be extended both for normal and toxaemia of pregnancy cases before any definite conclusion is derived.

130. Study of hypotonic inertia in labour under Dr. D. L. Poddar at the Nilratan Sircar Medical College. Calcutta.

The work was commenced on 8th August, 1958 and the abstract of the report of work done up to 30th September, 1958 is presented here.

The main object of the study was :—

(1) To find out the possible ætiological factors in uterine inertia and

(2) To evaluate diffierent methods of treatment for its control.

Cases were selected after :

(1) Surveying all labour cases with special attention to those in whom labour is expected to be prolonged for more than 24 hours.

(2) Assessing the behaviour of the uterus clinically by noting :

(a) the consistency of the uterus during contraction relaxation phases and,

(b) the intensity, duration and frequency of each contraction.

(3) corroborating the clinical assessment with instrumental methods using Tocometer and Tocograph ; Tocometer giving information about the resting tone and Tocograph recording the intensity, duration and frequency of each contraction as well as the type of relaxation.

Cases with diminished intensity, duration or frequency of contraction and a low resting tone (below 8 m.m.) were included in this series.

A detailed history was obtained and careful examination (including radiological examinations where indicated) was carried out with a view to elicit the possible aetiological factor or factors.

Different methods of therapy were tried either singly or in combination, and the effects on uterine contraction were studied individually by instrumental methods. The effects on the mother as well as the infant after delivery, were carefully noted.

Altogether 140 cases were selected from a total of 1,235 deliveries during the period of study but the treatment was carried out on only 109 cases, while the other 31 cases served as control.

Analysis of Clinical Data.

Incidence.—140 cases of Hypotonic Uterine Inertia in a total of 1,235 deliveries, constituted an incidence rate of 11.3 per cent.

Other features analysed were

1. *Age incidence.*

Below 20 years ...	47 cases	
20 to 30 years ...	... ,,	
Above 30 years ...	... ,,	9.3 per cent

Elderly patients constituted only 9.3 per cent of the series.

2. *Relation to Parity :*

Para 1	80
Para 2 to 5	50
Para 6 & above	10

42.8 per cent of the cases were multipara and 57.2 per cent primipara. Elderly primigravida were only 2 per cent.

3. *Period of Gestation :*

Below 40 weeks	64	
40 weeks	54	
Abovo 40 weeks	22	(15.7 per cent)

45.7 per cent had premature labour and 15.7 per cent postmature labour.

4.	Emotional factors (Nervous, hysteric)	20
5.	Toxæmia	24
6.	Postmaturity	22
7.	Occipito-posterior position	18
8.	Malpresentation	3
9.	Disproportion	3
10.	Twins	6
11.	Hydramnios	10
12.	Premature rupture of the membranes	10
13.	Induced Labour	5
14.	Antepartum hæmorrhage	2
15.	Post History of Caesarean Section	3
16.	Relative infertility	11
17.	Dystocia dystrophia syndrome	1

Idiopathic Group : In 47 (33.5 per cent) cases no apparent abnormality was found. Twenty seven cases out of these 47 were primigravidæ.

Combination of Factors : In 29 cases more than one factor was present.

ASSESSMENT OF THE EFFECTS OF DIFFERENT METHODS OF THERAPY.

The methods of treatment adopted and the results obtained, are shown below :—

Methods of therapy	Number of cases treated	Results: Good	Fair	Poor
Pethidine only	34	3	26	5
Pitocin only	12	10	2	-
Pitocin & Pethidine	12	12	-	-
* D.H.E.	18	13	2	3
** A.R.M.	13	7	6	-
A. R.M. & Pethidine	13	11	2	-
Oblivon	7	-	4	3

* D.H.E.—Dihydroergometrine.

** A.R.M.—Artificial rupture of the membranes.

From the above it is seen that intravenous pitocin followed by pethidine gave very satisfactory results. The cervix dilated quickly and the labour was shortened, and there was no untoward effect either on the mother or the baby.

Dihydroergometrine (D.H.E.) gave very satisfactory results in cases where the labour was not progressing well, with the cervix partially dilated and the head engaged. An intramuscular injection of .25 mg. D.H.E. produced dramatic results, delivery was achieved in a short time with satisfactory maternal and fœtal results.

In cases where stimulation therapy with pitocin or D.H E. were found unsuitable and the cervix more than half dilated and the head engaged, artificial rupture of the membranes (A.R.M.) was performed. The results were encouraging.

COMPARISON OF MATERNAL AND FOETAL RESULTS,

The maternal and fœtal results in the treated group are compared with those on the control.

Group of cases.	*Maternal mortality*	*Maternal morbidity*	*Caesarean section rate.*	*Corrected foetal mortality.*
Therapy group (109)	Nil	2.7 per cent	1.9 per cent	Nil
Control group (31)	Nil	16.1 per cent	6.5 per cent	6.5 per cent

This shows that the maternal and fœful outcome is better in the treated group.

1. Enquiry into the cytological and cytochemical behaviour of human placenta and its possible role in toxaemias of pregnancy under Dr. Chinmoy Ghose and Dr. Jyotirmy Chatterjee at the Calcutta National Medical Institute, and Institute for postqrriwuate Medical Education & Research, Calcutta.

The study was restricted to histochemical findings of alkaline phosphatase in human placenta and stained after standard method.

Study of 29 placentæ from normal pregnant women having spentaneous termination of pregnancy during the last trimester formed the basis for comparison to 54 placentæ from the toxæmic mothers. Clinically different types of toxæmia were considered according to standard classification. Qualitative and quantitative assessment of stain intensity were determined.

The results indicate that there is a variation in the amount of alkaline phosphatase, significantly increasing in the later weeks of normal pregnancy with a slight fall during the last week. In the toxæmia group the rise is significantly higher compared to the normal. Age, parity and the type of toxæmia did not influence the picture but the alkaline phosphatase content varied directly with the degree of toxæmia.

132. Studies of Rh-isoimmunization during pregnancy and its bearing on the incidence of erythroblastosis foetalis under Dr. S.C. Bose at the Medical College, Calcutta.

During the last five months 422 samples of blood from mothers, just after confinement, were examined for blood group and Rh antigen. In this series 5 samples belonged to Rh negative type. Similarly samples of cord blood from all the babies born of these mothers and 47 samples of husbands' blood were tested for blood group and Rh. In samples of cord blood, 7 belonged to Rh negative, and all the husbands belonged to Rh positive groups.

The direct Coombs' test was done on 400 samples of cord blood. The test proved negative in all.

133. Investigation into the causes of abortions in Guntur under Dr. R. Satyabhama Reddy at the Medical College, Guntur.

METHODOLOGY.

All the out-patients and in-patients in the Gynæcological wards of the Government General Hospital, Guntur with either a present or past history of abortions are being investigated with a view to elucidate its ætiology. The incidence of abortions in Guntur is relatively high (22 per cent) compared to that in Madras (20 per cent) and two western countries (10 to 15 per cent).

Complete history was elicited regarding the menstrual cycle, marital relationship, any attacks of fever, exposure to venereal disease, previous surgical intervention for general and gynaecological, causes and occurrence of tuberculosis or diabetes in the family.

Some laboratory investigations such as hæmoglobin percentage, urine for suger, blood grouping with particular reference to Rh factor, serum proteins and V.D.R.L. were carried out as a routine and wherever indicated, blood cholesterol and B.M.R. were determined.

A thorough check-up of the general condition of the patient with reference to disorders, either acute or chronic, pertaining to heart, liver or kidney was carried out. Local examination was also done to find out whether there was any evidence of mal-development of genital tract, displacements, tumors, or signs of old trauma.

Similar examination of a control series will be undertaken covering normal cases of pregnancy with no history of abortions.

RESULTS.

So far only 39 cases have been studied. A preliminary analysis of these cases shows that fevers are directly or indirectly responsible for the onset of abortion in about 30.7 per cent of the cases. Trauma to gevetalia found in about 15.4 per cent of the cases, comes next in order. Evindence of exposure to syphilis is noted in about 12.8 per cent.

The general nutritional status was considered to be normal in two-thirds of the cases examined and haemoglobin percentage was with in accepted limits in 74.3 per cent of cases. Since the work is in progress, detailedanalysis will be submitted at the conclusion of the investigation.

Four Rh negative mothers delivered Rh positive babies. The direct Coombs' test of the babies cells and indirect Coombs' test on the mothers' serum were negative. No evidence of Rh iso-immunisation in the Rh negative mothers was obtained.

In the above series, 41 mothers of group 'O' delivered 10 group A, 26 group B and 5 group AB babies. The direct and indirect Coomb's tests of baby's cells and mother's serum respectively were found to be negative. No evidence of A,B,O iso-immunization was detected by the direct and indirect Coomb's tests.

134. Enquiry into the incidence and nature of worm infestation in infants and pre-school children in Calcutta under Dr. Muktha Sen at the All-India Institute of Hygiene & Public Health, Calcutta.

The problem is tackled in two ways; (1) Cross-sectional study to provide information on the incidence of worm infestation in children below the age of 5 years, and (2) Longitudinal study of about 100 children from early infancy to the age of 5 years at intervals of 3 months.

So far, 428 children have been included in the cross-sectional study and 78 in the longitudinal study. The stool examination is done on all these children and in some of the cases hæmoglobin content of the blood is also estimated.

As the subjects for the enquiry were taken by random sampling it is expected that a picture of the problem of worm infestation in this age group would be available for the urban community of Calcutta.

135. Enquiry into the incidence and nature of infestation in worm infants and pre-school group of children in Indore under Dr. J.N. Pohowalla at the M.G.M. Medical, Indore. College

Till the end of September, 1958, 401 children below the age of 5 years, drawn without any attempt at selection from the out-patients and in-patients at the M. Y. Hospital and from the Sanyogitaganj Child Welfare Centre, have been studied at the Pædiatric Department, M.G,M. Medical College, Indore. After obtaining personal family, dietetic and social history, each case was physically examined. Fæces of every case was examined macroscopically for adult worms and microscopically for ova (i) under saline preparation and (ii) by concentration method. In most cases ova of thread worms have been looked for from the perianal area using transparent scotch tape. The work is in progress.

MENTAL HEALTH

136. Enquiry into the psychological factors related to adolescent adjustment under Dr. T.K.N. Menon, Dean, Faculty of Education and Psychology, M. S. University of Baroda, Baroda.

This research project was undertaken to explore the relative influence of relevant individual and environmental factors on social, emotional and family adjustments of young persons, ranging in age from 13 to 19 years. The nature and extent of occupational orientation of adolescents is also being studied.

In order to assess the degree of adjustment in these areas, three inventories are being standardized. Standardization work concerning Social Adjustment Inventory has been completed. Further work is being done to study social adjustment in relation to age, sex, achievement and rural-urban background. Emotional and Family Adjustment Inventories have been developed and are being tested.

A procedure to study general adjustment, namely Incomplete Sentences Test, has been developed and its utility is being tested.

An Interview Schedule with four parts has been prepared for use in determining the kind and degree of occupational orientation of adolescents. In connection with the study of occupational preferences, classification scheme has been developed. It is ready to be put to a reliability test. This instrument is necessary for studying the nature and implications of vocational aspirations of adolescents.

After preliminary trials a personality test has been developed. It is ready for reliability and validity studies.

Exploratory work has been done with regard to attitudes of adolescents. Specific items of the attitude test have been devised and some processing needs to be done before putting them in their final form. The next step would be testing and standardization.

Adolescence is characterized by weaning from parental protection and assertion of independence. In this context, it was thought to study the traits of dependency and self-sufficiency of adolescents. Review of relevant literature and item-construction are being done to develop tests for these two traits.

Many researchers have recognised the influence of socio-economic background of families on different aspects of behaviour. It was considered necessary to develop a scoring scale for measuring the socio-economic status of families and thus determine the behaviour of youth coming from upper and lower classes. Some survey work has been completed in Baroda in order to devise techniques for measuring socio-economic status. The results of the field survey are being used in evolving a scoring scale.

Efforts are being mainly directed towards preparation of necessary techniques for measuring various aspects of Adolescent Adjustment.

137. Enquiry on electro-encephalographic and electro-corticographic studies with the help of spceially designed magnetic pick-up wave analysers under Dr. M.V. Govindaswamy and Shri R. L. Narasimhaiya at the All-India Institute of Mental Health, Bangalore.

1. Electro-encephalographic recordings are being taken of patients referred to the department of E. E. G. and Biophysic of the Institute. In addition to the resting records, the patients are subjected to hyperventilation and photic stimulation, and the records were taken under these conditions in order to study the effects of these agents on the E. E. G.

2. A card has been designed and made for recording E. E. G. data in a form convenient for later analysis.

3. Records of normal subjects are being obtained to form a control group.

4. A magnetic recording drum has been designed and got made in a local workshop. The drum will be used for recording the effects of various stimuli on E. E. G. waves.

5. An electronically regulated powerpack and an amplifier have been designed and assembled in the laboratory for use with the recording drum.

138. Enquiry to establish the validity and reliability of sedation threshold test as defined by Shagass in diagnosis and prognosis of certain psychiatric entities under Drs. N S. Vahia at the Seth G.S. Medical College, Bombay.

This work was taken up with a view to test the validity of Shagass test for Sedation Threshold. As in psychiatry, at present, objective criteria of diagnosis, prognosis and treatment are not available, it war th ught that if Shagass Test is found useful as an objective test, the clinical work in psychiatry will be on a much more sound and scientific basis. Shagass has done a good amount of work in this direction and his claims are that Sedation Threshold Test, as carried out by him, can help in differentiating certain psychiatric conditions as well as in prognosticating and determining the indication of treatment.

MATERIAL AND METHODS.

At first, complete data such as age, sex symptoms and history of the patient was taken. Also the physical examination was dond. Weight and blood pressure were also taken.

A routin E.E.G. of the patient was taken before hand. The Sedation Threshold Test was done preferably on the next day, The necessary requirement of the test was explained to the patient. During the test the patient was made to lie down on bed completelly relaxed, the E.E.G. instrument being in anotner room. The patient was interviewed for about 15 to 30 minutes by Dr. N.S. Vahia and Dr. V.N. Bagadia, for the assessment of tention clinically. Venepuncture was done and for one minute after the venepunoture a brief control E.E.G. was taken. one c.c. of Sodium Pentothal was injected every 4o seconds. The solution which was used was prepared in such a way that patient was getting .5mg- of Sodium Pentothal kilogrom of body weight per 40 seconds. The Patient was asked to pronounce some difficult words like "British Conrtitution", "Chakravarti" "Municipalty", etc. after 25 seconds of injuction of 1 c.c. solution each time and it was noted whether he pronounced correctly or slurred- The point, that is the amount of c.c. at which the speech was slurred, was noted and the injuction was stopped after injecting one or two c.c. more. In some cases the point at which patient went to sleep also was noted.

As one could not get the end point sharply, in a few cases the solution was prepared as above but a double dilution was made to obtain the end point more satisfactorily. But as this method was found to be unsatisfactory, it was discarded. Then the procedure was modified further and the dilution was made 1½ times. This plan also did not work properly. So again the original plan was adopted.

In the beginning, for a few cases, injection Atropine Sulphate Grain 1/100 was injected intramuscularly to the patient before starting the test as we were using Sodium Pentothal instead of Amytal. This caused dryness of throat and it was difficult to distinguish whether the slurring point obtained was due to the dryness caused by Atropine Sulphate, on due to the effect of Sodium Pentothal. For this reason injection of Atropine sulphate before the test was discarded after the

proper consultation with physicians and anæsthetists. Exclusion of Atropine sulphate improved the results without any complications.

After doing the above procedure plotting of the curve was done as follows :—

Wave amplitudes of bifrontal tracings were measured. The amplitudes of all waves from 15 to 30 cycles per second, occuring in sequence of two or more, were measured. Also, the samples measured were those that contained the maximum fast activity during certain specified time period. In case of uncertainty more than one reading was taken and the mean was taken. The samples for measurement was taken from 25 to 45 seconds and from 0 to 10 seconds of the next 40 seconds period. Measurement which were taken in milimeters were then transformed in—Microvolts and graph plotted.

The curve was going up abruptly in the beginning till the slurring point was reached. Then it started becoming flattened out. These types of curves were found when there was no dilution of the solution.

In all, 25 cases were studied during the last 3½ months. The cases selected were schizophrenics, chronic anxiety state with somatic manifestations and hysteria.

As regards the sex, 22 males and 3 females were selected. The age varied from 14 years to 50 years.

The following tables show the age group and the classification of the diagnosis :

Table (I)

Age	No. of cases
10—20 years.	2
20—30 years.	15
30—40 years.	5
40—50 years.	3

Table (II)

Sex	No. of cases
Males	22
Females	3

Table (III)

Diagnosis	No. of cases
Schizophrenics	5
Anxiety Reaction with somatic manifestations.	5
Hysteria	1
Chronic Anxiety Reactions.	14

Treatment

The treatments given were as follows :

(i) E.C.T.	for Schizophrenics.
(ii) Insulin	for Schizophrenics.
(iii) Co_2. Gas	for Anxiety Reaction and Hysteria
(iv) Tranquilizers.	

Results

The tension (clinically) was from one plus to four plus which correlated with the tension in the curve (E.E.G.) in 20 cases. The slurring of speech clinically correlated with the slurring in the curve (E.E.G.) in 18 cases.

Nature of sedation curves

Following patterns were obtained :

(1) Curves with a sharp rise till the slurring point was obtained and then running in more or less a straight line.

(2) Same as above up to the slurring point. Then a straight line but afterwards again a sharp rise.

(3) Curves rising sharply, not a stretch but in the form of small waves and then after a slurring point is reached going down in the form of more or less a straight line also in the form of waves.

(4) Curves with gradual rise and looks almost like a straight line from the beginning to end. This happened in cases where the Sodium Pentothal solution was diluted double and even half times.

(5) Curve going up and up with slurring point mark but not falling down after the slurring point.

(6) Curve running the form of waves haphazardly with no relation to slurring point or any other factors (this happened especially in double dilution).

Remarks

Although the work was taken up with a great enthusiasm, the work could not be carried out because of lack of a Research Assistant and therefore no definite conclusion was made.

In the majority of cases in which the investigation was done, a definite correlation was found between the slurring point noted clinically and as determined by E.E.G.

There was also a definite correlation between the clinically manifested anxiety as judged from the questionnaire, and the sedation threshold as studied according to Shagass test.

Evaluation of the test, so far its utility in prognosis and in selection of different treatments, could not be carried out according to the original plan, because of short duration of work and small number of cases done.

Our impression is that the test is useful in objective evaluation of severity of anxiety in various clinical psychiatric syndromes. If it is found on further study that the test helps in the prognosis, and treatment also as claimed by Shagass it would be useful in clinical psychiatry as there is scarcity of such objective tests in psychiatry. Further work along these lines is considered advisable when circumstances for carrying out this test are more favourable.

139. Pilot studies on mental morbidity in selected parts of Mysore State under Dr. M. V. Govindaswamy at the All-India Institute of Mental Health, Bangalore.

The survey has been planned to determine the incidence of mental morbidity including epilepsy and imbecitity and to study the associated socio-economic and cultural conditions.

Statistics concerning the distribution of mental ailments in Indian population, either rural or urban, are not available ; the sources of information relevant to the survey, such as school-records, court-records, jail-records, infirmary-records and exhaustive demographic details are also not available.

Initially, a work schedule was prepared to guide the various steps of the investigation. Various local agencies were contacted with specially prepared schedules to elicit the relevant information. In the preparation of the design of the experiment, experts in the country and abroad were consulted. But the information thus obtained was found inadequate, and hence a comprehensive survey of mental morbidity could not be taken up straightaway. Four alternative modes of executing the survey taking into account the available time, staff and other conditions were considered, (1) a study of the psychiatric population in Bangalore City ; (2) a study of incidence of mental morbidity in selected areas of Bangalore City ; (3) a study of the population indentified as psychiatric by local physicians and (4) a study of hospitalized patients and out-patients apropos a control group.

Of the alternatives, the first two could not be taken up for investigation immediately and it was decided to take them up at a later date. The third approach was given first attention. The purpose of this investigation was to arrive at the population that was identified as psychiatric by the physicians. In this study a representative sample of physicians was interviewed with a schedule. But due to lack of proper record keeping no useful information could be obtained. It was decided however, to request the physicians to keep a record of cases, which in their opinion had psychiatric involvement, for a period of six months. In the meantime analysis of the hospital records concerning the in-patient and out-patient population, was undertaken.

The case-records of discharged local patients, available in the Mental Hospital were scrutinized and analysed. About 1000 cases (from 1954 to 1957) were listed with a view to conduct an extensive follow-up study as a part of the survey. Besides this, 1166 addresses of out-patients residing in Bangalore were collected and distributed among the eight geographical zones of the city, for the follow-up study. A house-to-house visit was conducted and the patients or other informants were interviewed with the help of a schedule. This investigation reveals information about the problems of the mentally ill in the context of community living.

For the purposes of finalisation, the Schedule prepared to study the incidence of mental morbidity was tested on a small sample of out-patients. A random stratified (with reference to geographical zones) sample of 200 cases was selected from among the 1166 out-patients

residing in Bangalore. The schedule was responded to by only 92 out-patients. This constituted 46 per cent of the chosen out-patient population.

The schedule was later revised bearing in mind the results of the study and also suggestions from Prof. K. B. Madhava. The revised schedule consists of two parts. The first has two sections: one eliciting information about the demographic details and also about significant genetic loading in patients suffering from mental illness and the other containing items pertaining to the physical surroundings of the family, employment and financial conditions, the problem of immigration, neighbourhood conditions, socio-economic, cultural and religious factors, and the influence of industrialization and urbanization on the family. The second part deals exclusively with the patient's activities, his adjustments, obligations, hobbies and interests, ambitions, achievements, conflicts and frustrations.

In the revised schedule items likely to furnish purely subjective impressions are reduced to a minimum; the items that were found to be ambiguous were eliminated; relevant items referring to different patient populations were included under separate sections. The length of the revised schedule was greatly reduced (from 149 items in the original schedule to 78 on an average in the revised schedule), without, however, omitting any significant item.

The revised schedule will be administered extensively to the out-patient and discharged in-patient population in Bangalore.

140. Study of relationship of child rearing practices or antecedents to the behaviour problems in children under Dr. B. D. Bhatia, Director, Child Guidance Clinic, College of Nursing, New Delhi.

Earlier, research conferences were held with a view to (1) planning the design of investigation, (2) deciding about the objective techniques to be used, and (3) laying down the hypothetical variables to be explored in the study.

It was also decided that the term 'behaviour problems' should be used in a broad sense so as to include both *personality and conduct problems* and that the nature of the study should be *experimental rather than exploratory*. As such two groups of children will be selected at random, the 'Experimental Group' and the 'Control Group', each comprising 100 children between the ages of 5 and 15 years.

It was decided to match the children of the two groups in the following aspects as far as possible (a) Sex, (b) Age, (c) Socio-Economic Status, (d) Educational Status.

It was decided to collect the data pertaining to our study by interviewing parents of children of the two groups. For this purpose an interview schedule, consisting of 124 questions, has been formulated. It has also been decided that the interviews be supplemented in terms of parents' spontaneous verbatim reports about some of their experiences with regard to certain child rearing practices and children's reactions to them.

It was also decided to have 12 hypothetical child rearing practices for an intensive study. They are (1) Pre-natal practices and attitudes towards pregnancy, (2) Feeding practices, (3) Toilet practices, (4) Sex and modesty training practices, (5) Sleeping practices, (6) Sibling relationships, (7)Sex preferences,(8)Practices regarding the authority and status of adults & parents, (9) Practices regarding toys and play, (10) Practices regarding school studies, (11) Practices regarding personal demands of the child and (12) Discipline Punishment and reward.

The interview schedule will be modified and finalised to be used in the regular investigation after pre-testing. Pre-testing covering about 40 interviews with the parents of both 'experimental and control group' children has been completed by the end of November 1958.

141. Nutrition Research Unit under Dr. B.C. Guha at the University College of Science and Technology, Calcutta.

I. Studies on the biosynthesis of ascorbic acid

An enzyme preparation has been obtained by ammonium sulphate fractionation of goat liver extract (after sedimentation of the nuclear and mitochondrial fractions) which converts D-glucuronolactone into L-ascorbic acid in the presence of potassium cyanide (0·05M). The rate of synthesis decreases with decreasing cyanide concentration. The enzyme preparation also converts L-glucuronolactone in to L-ascorbic acid and in this case addition of cyanide or other factors is not necessary. Sodium salts of the free acids corresponding to the lactones, as also other substrates tried are ineffective. The conversion from the lactones does not require any added co factor like DPN, TPN, ATP,AMP, adenine,UDP, nicotiamide $Mgcl_2$. CoA, glucose-1-phosphate, glucose-6-phosphate, FAD, FMN, etc. and is, in fact, inhibited by UTP. P.C.M.B. inhibits this synthesis, the inhibition being reversed by GSH indicating that the enzyme contains some-SH groups. The biosynthesised ascorbic acid has been identified both chromatographically and biologically.

The liver and kidney tissues of a number of different species of animals selected according to phylogenetic evolution have been examined in regard to their capacity for synthesising ascorbic acid in presence of of cyanide. The results obtained reveal some interesting features of a biochemical aspect of evolution.

II. Studies on the isolation of bound ascorbic acid (ascorbigen) from cabbage

A sample of ascorbigen has been isolated from cabbage by gradient elution method on a laboratory scale. It appears to be pure and is under further study.

III. Biochemical studies on the bound form of nicotinic acid (niacinogen).

'Niacinogen' has been isolated from rice and wheat in a pure crystalline form. The molecular weight has been found to be approximately 12000. The peptide moiety contains seventeen amino acids. Some other physical and chemical properties have also been determined.

IV. Studies on hemoglobin and iron metabolism.

Electrophoretic studies have been further extended to other species of birds and to one species of the flying mammal, the 'bat' (*Chiroptera Nyotis*). The hemoglobins were analysed by the different electrophoretic techniques, *viz*, paper electrophoresis, agar electrophoresis and starch electrophoresis. The results obtained by these techniques confirmed those reported last year.The electrophoretic pattern of bat hemoglobin had shown identity with hemoglobin E and not with A or any of the other avian hemoglobins. Some of the avian hemoglobins were subjected to alkaline denaturation and ion-exchange chromatography using IRC-50.

142. Nutrition Research Unit under Dr. G. K. Gokhale at the Seth G. S. Medical College, Bombay.

(A) Studies on Human Subjects.

I. Nicotinic acid metabolism in health and disease :

(Inter-relationship between the blood PN levels and haemopoiesis and/or hepatocellular functions)

The fluctuations of blood cell Pyridine Nucleotide level in progressive and retarded haemopoiesis were reported last year and their relation to haemopoietic process was suggested. A possibility, however, existed that those fluctuations in PN levels might also be related to the hepatocellular functioning during anaemia, since liver damage of varying degree has often been encountered in anaemias. Work was, therefore, undertaken to observe whether the PN metabolism is primarily connected with the haemopoiesis or whether the hepatocellular functions play a more dominating role.

II. Iron matabolism in health and ntsease :

On the recommendations of the Nutrition Advisory Committee, the work on plasma iron fractions and copper was re-started. The work has been channeled to throw further light on the PN metabolism during anæmias and its possible relationship with the iron metabolism in that condition.

It has been observed that the blood cell PN levels increased both in anæmia and cirrhosis of liver, with mild anæmic condition. Attempts are being made to isolate the effects of anæmic and those of liver damage by studying cases exhibiting these two conditions independently.

(B) Experinental Studies.

In order to correlate the clinical findings, experimental work has also been undertaken to establish the influence of hepatocellular functions on PN metabolism by inducing liver damage in experimental animals, viz., albino rats.

143. Enquiry on the role of nutritional deficiencies in the causation of peptic ulcer under Dr. C. Raghavachari at the Medical College, Trivandrum.

About 410 cases have been studied (224 at Madras and the rest at Trivandrum) intensively from the dietary, clinical and biochemical aspects.

Age: Seventeen to 70 years. 75 per cent are between 30 and 50 years. 5 are females and the rest males, 20 per cent live in city and the rest in villages.

Social status: Ninetyfive per cent have income varying from Rs. 15 to 40 per month. 60 per cent of the cases are agricultural labourers.

Results of diet survey: Three hundred thirtyeight patients were investigated by "Questionnaire" method and 40 families by weighment method for their dietory habits and intake.

Diet: Staple food in most cases is cooked rice and tapica Through 95 per cent claim to be non-vegetarians, the amount of animal food taken is very low. Vegetables and fruits are consumed only when they are plentiful in supply. Milk and milk products are absent in their diets. Condiments and spices are consumed in large quantities. Irregular intervals between meals and missing a meal or two in 5 to 10 days are common.

Deficiencies: Quantity and quality of protein was low. Portein was deficient in 55 per cent and fat in 80 per cent. 88 per cent were deficient in vitamin A. 20 per cent were deficient in vitamin B_1. 92 per cent were deficient in vitamin C, and 26 per cent deficient in niacin. All were deficient in riboflavin. 93 per cent were addicted to tobacco (50 per cent heavy smokers). 25 per cent were addicted to alcohol. Operated and non-operated cases did not show much difference. The patients' diet when compared with controls did not show much difference except in spices.

Beo-chemical investigation.— Results of Fractional Test Meal Analysis. One hundred five operated cases, 305 non-operated cases and 25 cases of recurrent ulcer were studied.

Volume of resting juice.— Varied fron 10 c.c.—600 c.c. with only 3 per cent above 80 c.c.

Free acidity.— Only 33 showed free acid concentration higher than 50 clinical units. 2 per cent were cases of achlorhydria.

Starch.— In 80 per cent of cases starch was present in ¼ to 1½ hours' specimens.

Mucus.— Seventyfive per cent cases showed no mucus in any of the specimen.

Bile.— Eightyfive per cent cases were negative in all specimens.

Blood.—Positive in 12 per cent of samples other than resting juice. Positive in all specimens of gastric carcinoma only.

Residual juice.—Varied from 0 to 400 c.c. It was above 100 c.c. in only 4 per cent.

Ninety-five of the cases studied were duodenal ulcers. In view of the above results, it was difficult to establish any positive correlation between hyper acidity and duodenal ulcer.

Serum total proteins.—(By Microkjeldahl Method) 163 cases were studied, of which 25 were controls, 64 operated cases and 74 clinically diagnosed as ulcer but not operated.

Average total proteins for control cases	..	7.47 per cent
,, operated cases	..	7.03 g. ,,
,, non-operated cases	..	7.13 g. ,,
Serum albumin average albumin for control cases	..	4.44 g. ,,
,, operated cases	..	4.14 g. ,,
,, non-oprated cases	..	4.18 g. ,,

Electrophoretic pattern.—Fifty per cent of the 89 cases studied showed a prominent B=globulin band indicating a rise of lipo-proteins.

Vitamin C.—Fifty per cent cases were studied. Sixty per cent after ulcer cases had values below normal.

Serum total lipids —Total cholesterol, phospho lipids and iodine number were investigated. The results are :—

	Total lipids.	Choles-terol.	Phospho lipid.	Iodine number
Average for control cases ..	614	246	5.51 mg. per cent	114
Average for operated cases ..	559	217	4.18 ,, ,,	56

For serum lipids, number of cases studied were only 16 controls and 37 operated cases.

144. Goitre Piolt Survey Project, Pathankot, Punjab.

The Survey work undertaken towards the end of last year in Nurpur Tehsil of Kangra District and Dasuya Tehsil of Hoshiarpur District was completed in the year under report. Besides this, some of the villages of Pathankot and Gurdaspnr Tehsils of Gurdaspur District which could not be covered during the last year were also surveyed.

During the whole year, 127 villages were visited and 4,540 families comprising of 20,641 individuals were examined for thyroid enlargement amongst the general population. Out of these, 10,040 individuals showed thyroid enlargement of varying degrees, thereby giving an incidence of 48.6 per cent.

Similarly, 16,101 school children were examined. The goitre incidence varied from 25 pea cent to 65 per cent in them.

Some of the villages of Dasuya Tehsil of Hoshiarpur District which are situated close to the river Beas and Shivalik range of Hills showed the incidence of thyroid enlargement of about 72 per cent.

In Nurpur Tehsil of Kangra District the incidence in some villages amounted to 88 per cent.

During the surveys in five different local schools the goitre incidence was found to be 75.6 per cent amongst school children.

The team carried out estimation of hæmoglobin in 5,860 individuals during the year.

Sixty-three samples of drinking water were collected from the places which were surveyed for thyroid enlargement and sent for analysis. Similarly 26 samples of salt collected from the various shops in the experimental zones of Kangra District were sent for analysis.

145. Scheme on relationship between malnutrition and bladder stones under Dr. D. A. Anderson at the Evange line Booth Hospital, Ahmednagar.

A clinical study has been made of all primary bladder stone cases in the District of Ahmednagar admitted to the Evangeline Booth Hospital during the years 1951 to 1957. Bladder stones secondary to known obstruction to the urinary tract have been excluded.

Incidence.—During this period 103 cases of primary stone in the bladder have been admitted for treatment. The incidence in the District varied from 0.3 to 0.85 per 10,000 of population (total population 140,000) annually, being more in famine and semi-famine years. This is lower than the average incidence worked out by McCarrison and Ranganathan.

Location.—The number of bladder and urethral stones exceeded the number of upper urinary tract stones by about 4 : 3 in the hospital records, while in an American series the corresponding proportions were 1 : 5.

Age.—The maximum incidence of bladder stones in the series was in the age group 5-6, years, 59.4 per cent were under 10 and 68.7 per cent under 15 years of age.

Sex.—The sex ratio was, 97.1 per cent male and 2.9 per cent female.

Occupations.—The patients were from families with rural occupations in 93 of per cent cases, while, in general, the rural population accounted for only 57 per cent of the hospital in-patients. A great majority of patients or their parents were Maratha farmers, farm labourers or artisans.

Clinical.—The clinical picture was one of bladder irritation and not obstruction unless the stone was impacted. There was no evidence of any congenital abnormality found in the series. Recurrences were vitamin relatively rare, and serious infection was uncommon.

Nutrition.—The nutritional status of the patients was poor corresponding to their poor economic background, but in 1957/58 sever degrees of vitamin A deficiency were not observed in stone cases studied.

B. (1) *Biochemical.*—Low vitamim A and carotene levels were obtained in the the blood of stone cases studied.

Blood calcium levels were obove normal (35 per cent have over 10.6 mg. per cent). Blood calcium levels were normal.

Urinary Calcium excretion for 24 hours was low, and this was related to the low values of calcium found in the diets consumed by patients suffering from bladder stones.

Urine phosphorus excretion was also low, which contrasted with the high levels of phosphorus intake by patients suffering from bladder stones. However, dietary studies, were made under village conditions, while the urinary excretion studies were carried out in hospital as in-patients ; and

so, the excretion of calcretion and phosphorus needs to be repeated under normal village conditions.

(2) *Histological studies and stone analysis* :– These studies are being carried out at the Nutrition Research Laboratories, Coonoor.

C. (1) *Diet surveys* .— Preliminary diet surveys of the cases of bladder stones and the village groups from which a great majority of these cases are drawn show the following :—

(1) High protein

(2) High calories

(3) High phosphorus intake

(4) Low calcium intake

(5) Low vitamin A and carotene intake

(6) Low vitamin C intake

The high protein and calorie values are due to the large amount of millets (*Bajra and Jowara*) eaten by the villager, and this also accounts for the high phosphorus intake.

Calcium intake .— So far, adequate studies on the hardness of water have not been possible, and so the total calcium intake is not yet determined ; but it is definitely not high in the food alone.

The low vitamin A, carotene and Vitamin C values are due to the lack of fresh fruit, vegetables and milk products in their diets.

C. (2) *Climate.*—The average rainfall in this District is 15 to 25 inches ; the average maximum temperature in May is 101.9° F. and the average minimum in December is 52.9° F. The humidity is low.

As the village community spends a great deal of time in the open fields the fluid balance and related concentration of urine need further consideration. Preliminary studies have been undertaken.

146. Enquiry on the influence of soil condition and genetic make-up on the yield and nutritive value of Indian pulses under Dr. G. C. Esh and Dr. U. P. Basu at the Bengal Immunity Research Institute, Calcutta.

Chemical analysis.—Eighty-five pure-bred and mature samples of nine varieties of pulses from 1957 crop, collected from different State Agricultural Farms and Research Institutes were analysed for their protein content. From the crop of 1958, eighty-six samples, mainly from same localities and of the same strains were collected, and thirty of them were analysed.

The significant findings regarding the distribution of protien in 85 samples of 1957 crop are summarised below :

(a) The results indicated a wide variation in the protein percentage of pulses when grown from different genetic strains and under different soil and climatic conditions.

(b) When same strain pulses were grown in different localities a variation ranging from 13 to 33 per cent in the protion content was observed showing the influence of environment on the composition of pulses.

(c) Similar variation ranging from 11 to 37 per cent depending upon the nature of the strain was observed when pulses like Bengal gram, red gram and black gram were raised in the same locality showing the influence of genetic constitution on the protein level.

(d) Larger variation to the extent of 60 per eent in the protein percentage of Bengal gram and red gram was observed when both strain and environment varied.

Analysis of thirty samples of 1958 crop showed that the protein content of the samples except in a few cases were in good agreement with that of the previous year's samples.

The limited data collected till now did not show any correlation between the yield and total protein content of pulses.

Digestibility and Biological Value—(By balance sheet method) :—

The limited data collected till now about the digestibility and biological value of pulse samples with high and low protien contents indicated the following :—

The digestibility slightly increased and biological value slightly decreased with the increase of protein content of Bengal gram grown from different genetic strains and environmental conditions. It was interesting to note that in case of red gram, green gram and lentil, both the digestibility and biological value tended to increase along with rise in protein content under the influence of both genetic and environmental conditions.

Growth Experiments .—Weanling rats were fed high protein and low pro tein Bengal gram at diets 12% protein level to observe th growth promoting influence. The data from preliminary experi ments showed the following trends :

(a) High protein Bengal gram when fed in raw stote at 12 per cen protein level showed slightly higher protein efficiency ratio.

(b) When fed along with wheat supplement (so as to supply 5 pe cent protein in diet) both high protein and low protein Benga gram, obtained from same strain but from different localities maintained equal protein efficiency ratio.

(c) When casein as protein supplement was added to diets con taining a constant proportion of Bengal gram of high and lo protein content, equalising the protein content to 18 per cent the results showed superior nutritive value of high protein puls

147. Enquiry on separation of different forms of vitamin A_2 and effect of replacement of vitamin A by vitamin A_2 in small land animals under Dr. P. D. Dalvi at the S. M. S. Medical College, Jaipur.

Work was continued on the compound showing absorption at 325 mμ. its properties were studied in detail, as also the work on the separation of different forms of Vitamin A_2.

Properties of 325 m μ. absorption compound :—

Anhydro vitamin A was prepared by the method of Shantz. Unsaponifiable material E 1% can 325 mμ. 1284, obtained after the saponification of vitamin A palmitate concentrate (1 m. i. u/g.) was used as the starting material. The material was refluxed for about 2 hrs. in presence of p. toluene sulphonic acid (which in no case should be less than 2 mg). The refluxed material was cooled, taken in petrol and then adsorbed on a suitable column of alumina weakened with 10per cent water. The band of weakly adsorbed anhydro vitamin A from the column was removed by developing the same with light petroleum. After repeated chromatography, the final product showed E, $1\%_{cm}$. 368 mμ. = 2210. The yield was much less than expected as no precaution was taken to collect the separated water formed during the dehydration process.

325 mμ. absorption compound was prepared by steaming ethanolic solutions (1-5 mg./100 ml. E 368 mμ.=2210) acidified with dil. HCl (pH 3.5) in pyrex ampoules for 20 min. The steamed liquid was cooled, taken in petroleum, and adsorbed on columns of alumina weakened with 10 per cent water. The best fraction of a flowing chromatogram, had $E_{1\,cm}^{1\%}$. 325 mμ.=1320. Examination of solutions of anhydro vitamin A steamed for different periods showed that it was converted to 325 mμ. absorption material through several intermediate compounds, of which one was with absorption spectra closely resembling rehydro vitamin A.

Effect of Steaming on vitamin A and anhydro vitamin A in acidic ethanol.—At one stage, both the unsaponifiable material and anhydro vitamin A, gave absorption maximum at 290 mμ.

Solvent partition.—The petroleum—83% ethanol distribution ratio for the whole oil was 96 : 4, while for the 325 mμ. compound and vitamin A alcohol the ratios were 37 : 63 and 40 : 60 respectively. These values approximate those of vitamin A fatty esters and free vitamin A respectively.

Dehydration.—The 325 mμ. compound could not be dehydrated by anhydrous alcoholic HCl, either N/10 or N/30.

Oxidation.—MnO_2 specially prepared and found quite reactive with vitamin A alcohol (in petroleum) did not oxidise the new compound, even to a slight extent. Irradiation :—Ethanolic solutions of the new compound (5 mg. to 7 μg/ml.) on irradiation gave reduced E values at 325 mμ., but the decrease was less rapid than in case of

similar solutions of vitamin A alcohol. These also showed a distinct maximum at 280 mμ.

Biological potency.—The experiments on vitamin A depleted rats indicated that the biological potency of the new compound was at least 50 per cent of vitamin A alcohol.

It appears that anhydro vitamin A is acted on by dilute acids to yield 325 mu. absorption compound, and during the process of convertion many compounds are formed, one of which is probably Shantz's rehydro vitamin A obtained by feeding rats on anhydro vitamin A. The rats could not convert the rehydro vitamin A further to 325 mμ. compound or vitamin A, but this convertion was possible by the action of acids. The 325 mμ. compound has biological potency equal to at least 50 per cent of that of vitamin A, and is probably an isomer of vitamin A alcohol.

Separation of vitamin A_2 isomers from halibut fish oils.—Due to non-availability of fresh water fish oil concentrates or oils, halibut oils from Norway (30000—50000 i. μg.) and of Crookes brand (30000 i.u./g.) were saponified in the usual way and the non-saponifiable factor was enriched by adsorbing the same on columns of alumina weakened with 5 per cent water. The enriched material 11.8 g. $E^{1\%}_{1\ cm.}$ 320-325 mu. 113, was used for repeated chromatography on alumina weakened with 5—10per cent water. Of the developed fractions those free from anhydro vitamin A and giving absorption maximum towards higher wave lengths were combined and repeatedly chromatographed. Finally, from the seventh chromatography two fractions were taken in spectroscopic petrol and their values read. These fractions gave max. 357 mu. and an ineflexion at 280–282 mu. unlike max. 352 mu. and 286 mu. (both peaks) observed for trans vitamin A_2. $E^{1\%}_{1\ cm.}$ value could not be determined on account of insufficy of the material. But judging from $E\ 1\%_{cm.}$ 350—355 mμ.=750 of the best fraction obtained from third chromatography it may be safely surmised that these fractions are nearly pure. Whether the isolated vitamin A_2 is one or a mixture of many isomers and, if it is one, whether it is comparable to neo-vitamin A, is a point yet to be determined. This requires separation of vitamin A_2 in good quantity, and the study of reactions with maleic anhydride, manganese dioxide, etc.

148. Physiological studies of human milk and its role in infant feeding under Dr. A. C. Majumdar and Dr. (Smt.) Amala Chaudhuri at the Institute of Child Health, Calcutta.

In continuation of the last year's work on the above scheme complete chemical analysis of a total number of 87 samples of human milk collected from Calcutta and its suburbs was made. These samples include milk at different periods of lactation starting from 3rd day up to 24 months. Socio-economic and nutritional data of each mother were collected. Milk samples from the first to the ninth month were regarded as mature milk, those up to 10 days after delivery as colostrum and those of lactation period after 9 months as late-milk. Lactation period 11th day to 1 month was termed as transition period. As the quantity of milk samples was small micro-analytical techniques were adopted as far as possible. The chemical analysis of the milk was carried out with reference to the following items, subject to the availability of sufficient quantity of milk. The figures denote the average values obtained for mature milk :—

(1) Specific gravity 1032.0; (2) Lactose 7.16 g.ser cent; (3) Fat 3.55 g. per cent; (4) Total protein 1.40 g.phr cent ; (5) Albumin 0.999 g.per cent ; (6) Casein 0.410 g.per cent ; (7) Non-protein nitrogen 0.503 g.per cent ; (1) Calorific value 67.8 Cal. per cent; (9) Chloride 154.7 mg. per cent ; (10) Inorganic phosphate 5.61 mg. per cent; (11) Total solids 12.04 g.per cent; (12) Solids-not-fat x.44 g. per cent (13) Ash 0.180 g. per cent ; (14) Sodium 5.4 m.Eq. ; (15) Potassium 6.4 m. Eq. ; (16) Calcium 29.15 mg. per cent ; (17) Magnesium 5.23 mg. per cent and (18) Ascorbic acid 1.88 mg. per cent.

The statistical analysis of the data will be done as soon as the analysis of some more samples of milk is carried out. Abnormal values obtained in a few cases of mothers with diseased children, are not included.

The data so far collected in the course of this investigation for the proximate principles in human milk, are in agreement with those reported in the Western countries. As regards the other items given above, the literature is scanty. However, the results of this investigation did not differ much from the reported figures. The potassium concentration in milk was found to be low, and the optimum ratio of Na : K (5 : 11) for the normal growth of infants was not found in the milk studied by us. Ascorbic acid value also did not differ much.

Attempts were made to classify the data to find out whether (a) age of the mother, (b) parity and (c) lactation period had any influence on the composition of human milk. It was found that the age of the mother had little influence on the composition of milk. But fat content of the milk from mothers of age group 31—40 years was high (4.8g.per cent) Total solids and ash content increased gradually with age, with a maximum of 12.58 g. per cent and 0.192 g.per cent respectively in the above age group. Though no variation could be observed in the composition of milk with parity, total protien value (1.21—1.66 g.per cent) gradually increased with parity. It was also noted that the composition of milk with reference protien was parti- cularly low in second para. Milk from 6th to 11 thpara had comparatively high values for total protien (1.66) and fat (4.7 g.per cent).

A comparison of the data on the composition of milk at different lactation periods viz., colostrum period, transition period, mature milk period and late lactation period, showed that specific gravity and total solids decreased, while lactose content increased gradually. Milk of transition period and late-lactation period had high values for fat (4.5 and 4.1 g.per cent respectively). As regards the albumin : casein ratio, the value was least in colostrum and maximum in mature milk. Maximum calorific value was observed during the transition period while it was lowest in the colostrum period. A good deal of variation was, however, found in the proximate principles in mature milk although the calorific value was nearly constant.

149. Enquiry on the mineral nutrition of lactic acid producing bacteria under Dr. D.B. Desai and at the B.J. Medical College, Poona.

Experiments were undertaken to investigate the influence of vitamins and amino-acids on the requirements of mineral ions of the lactic acid bacteria. In the first series, investigations were confined to a study of the effects of total absence or presence in small amounts of vitamins and amino acids on the requirements of L. arabinosus for K, Na or NH_4.

When the organism L. arabinosus was grown in a medium, devoid of riboflavin and folic acid, the concentration of Na at which the maximum growth was observed remained unaffected by the absence of these vitamins, though the actual rate of growth was less. Effects of the NH_4, ion on the growth at various levels of K were also unaffected by the absence of these vitamins.

L. arabinosus cannot grow in the absence of Ca-pantothenate and nicotinic acid. At low concentrations of these, the inclusion of Na in the medium was found to be of decided advantage. The fact that the effect was observed only at low concentrations of the vitamins, should be of considerable importance especially in the micro-biological assay of the concerned vitamins. However, with L. mesenteroides the effect was not so marked.

The effect of exclusion of certain amino acids like glycine, alanine and aspartic acid on the requirement of the various ions was also studied. In all the cases, improved growth was observed more with Na than with K. The same results were obtained at different levels of tyrosine and cystine which are required by L. arabinosus for growth.

The main point that emerges from these investigations is that Na as well as K can promote the growth of the lactic acid bacteria, though of course, the amount of K required for this purpose is very small as compared to the amount of Na. The role assigned to Na in the literature so far is only that of antagonist to K, i.e. K is essential for growth and Na inhibits it.

The second part was confined to the study of the effect of various ions on glycolysis of glucose. It was studied in the resting cells of L. arabinosus by determining the rate of production of lactic acid in terms of the amount of Co_2 liberated from bicarbonate, at pH 6·0 and 7·0, in the atmosphere of Co_2 and of air.

It was observed that K at concentrations higher than 2 mg./c.c. Na higher than 1 mg./c.c. and NH_4 at concentrations higher thad 0·25 mg./c.c., all inhibit glycolysis of the resting cells of L. arabinosus. This may be due to the osmotic imbalance caused by the high concentrations of the ions and may not be due to their influence on the functioning of any of the enzymes involved in glycolysis. Perhaps, at lower concentrations than those employed in this study, it may be possible that the latter effect namely that on the glycolytic enzymes may become apparent.

This was the picture obtained at pH 6·0 and in an atmosphere of air. The effect obtained at pH 7·0 was not significantly different from that obtained at pH 6.0, though the production of Co_2 at this pH was higher. Similarly, in the atmosphere of Co_2, no change in the picture was noted though here again, the production of Co_2 was higher than when the glycolysis was carried out in air.

150. Enquiry on malignant malnutrition in children under Dr. L.S.N. Prasad at the P.W. Medical College, Patna.

A comparative study is being made of the effects of vegetable protein diet (9·5 ounces of gram, 2 ounces of flour & 2 ounces of sugar; 1400 calories), and skim milk diet (6 oz. of skim milk powder, 2 ounces of sugar, 3 ounces of banana and 1 ounce of rice; 1100 calories) in cases of malignant malnutrition. Sixteen children suffering from the syndrome have been investigated so far. Majority of these cases usually presented themselves with low body weight (usually 30 to 50per cent of the expected weight), and changes in skin hair and eye. Some of them also showed orogenital syndrome. Marked improvement was noticed on both the diets. Anæmia present in these children required administration of iron; in the absence of added iron anaemia deteriorated markedly. It was also observed that the children took only 300 to 600 calories in the beginning due to marked anorexia. It is hoped that a proper assessment of the effects of vegetable diet and skim milk diet will be possible when the number of cases in each group is increased to about 50.

151. Study of articles of food, diet and dietary habits of various tribes of the North East Frontier Agency under Dr. S.R.K. Iyengar at the Health Training and Research Centre, Pasighat.

During the current year the work was mostly directed towards the study of Endemic Goiter, Cretinism, Deafmutism and Mental Retards. 27 Health Units undertook the work using a prepared proforma covering a population of 3,971. The incidence of Goiter varied from 0.9 per cent to 69 per cent in different areas.

2. An intensive study of Goiter, Cretinism, Deafmutism and Mental Retardation in relation to Blood Protein Bound Iodine was done among 18 families. The report was interesting, the percentage of hypothyroid state (55.1per cent) was more than the incidence of Goiter (47.2 per aent).

3. The Botanical Survey of India have identified many of the indigenous plants which are used as food articles by some of the NEFA Tribals.

152. Statistical analysis of the health records of pupils in Poona, between the ages 10 and 18 with special reference to their height and weight, and menarche in the case of girls, under Dr.(Mrs) Kamalabai Chitale, Poona.

(1) A scrutiny of the completed health examination sheets was begun in the month of June, 1958. Repeated measurements, particularly of height and weight were obtained on children from twenty schools, for four years: 1953-54 ; 1954-55 ; 1955-56 and 1956-57. This study was confined to pupils of ages 10 to 18 (both inclusive).

(2) Code card for each specified age covering all the items included in the health examination, was prepared. Data obtained on 16,828 girls were transferred on to these cards which were punched and verified. About 8,000 cards for boys had already been punched and part of these, verified. About 6,000 schedules are still to be coded and their cards punched.

(3) Tabulation of girl pupils' health data will be immediately started.

153. Studies on the role of dietary protein in the synthesis, enchancement or diminution, in the activity of pancreatic trypsin under Drs. R. C. Shukla and B. K. Malviya at the K. G. Medical College, Lucknow.

The first step in this investigation was to evolve a suitable operative procedure by which it would be possible to obtain the pancreatic juice in its pure form and yet keep the animal alive and healthy for a reasonable length of time. With this object in view, several operative techniques were given a trial but only the following method was found to give satisfactory results.

In this procedure, a part of the duodenum having the opening of the major pancreatic duct was isolated and made into a pouch into which a canula was fixed. A duodenal canula was also fixed so that the pancreatic juice passed back into the duodenum when not needed. If, however, the canula failed to function satisfactorily due to sloughing of the ponch sometimes, the remaining part of the duodenum was transplanted out with the opening of the pancreatic duct in its centre. The excess juice could be given back to the duodenum through the duodenal canula, when not required.

The successfully operated dog is under experiment; the tryptic activity of the pancreatic juice is being investigated under the influence of different diets.

154. Study on the urinary changes on changing the cereal in the diet from wheat to rice and vice versa under Drs. N. P. Banwari and K.S. Sharma at the G.R. Medical College, Gwalior.

The main object of the work is to elucidate the relative calculogenic effect, if any, of the cereals wheat and rice. Wilson & Mukerjee and in a later study Banwari & Sharma had shown that wheat in contrast to rice, brought forth certain changes in the quantity and concentration of urine which might favour formation of calculi.

That cereals might be implicated in some way in the production of urinary calculi was shown by McCarrison (1927-28) in an experimental study on rats. The latter studies have borne out his observations in a way in human beings too, yet the exact role played by cereals remains to be established.

In the present investigation, controlled study on two human subjects was carried out. Both the subjects, free from systemic disease, were admitted to the Ophthalmic Wards of J. A. Hospital, Gwalior. They were put on strict dietary regimen (Wheat-Rice or Rice-Wheat) over two consecutive periods of 10-days each. The diets were alike with respect to the non-cereal components and were sufficient as regards calories and proteins. In one case, the rice diet was followed by wheat diet, while the latter preceded the rice diet in the other case. Twenty-four hour urine was carefully collected, measured and analysed daily throughout the study. Quantitative estimations of calcium, phosphate and nitrogen where alternated with estimations of chlorides, creatinine and uric acid during the two dietary periods in the two cases. The day to day total water intake in them was also accurately assessed and recorded.

The total urinary output was much greater on rice than on wheat diet in one of the two subjects. In the other it was practically similar on both the diets.

The total quantity of calcium excreted in urine per 24 hours was greater on rice diet in one case and on wheat diet in the other, but the per cent concentration of calcium in urine was higher on wheat diet in both the cases.

Total 24-hours phosphate excretion was more on wheat diet in both the cases, though the percentage concentration was more on wheat diet in one subject and practically similar on the two diets in the other.

The 24-hour chloride excretion as also its concentration per 100c.c. of urine was practically similar on the two diets in one subject but higher on rice diet in the other case. In this case there was also much greater total urinary output on rice diet than on wheat.

Uric acid excretion in 24-hours as well as its per cent concentration in urine was much greater on rice than on wheat diet in both the cases.

155. Longitudinal studies of anthropometric measurements during the first two years of life in healthy Indian babies in Delhi State under Dr. Sheila Singh Paul at the Kalavati Saran Children's Hospital, Lady Hardinge Medical College, New Delhi.

The Scheme was started to establish norms on certain anthropometric measurements during the first two years of life. So far, no data of this nature are available for use in pediatric practice.

Two groups were selected. The first group consisted of 165 healthy new born babies, registered from 1st June to 30th September, 1958. Only those mothers who were willing to co-operate were included in the study ; hence the numbers were small. Data were collected at birth and every month thereafter.

The second group consisted of 36 one year old healthy infants registered during the same period.

Group I will be followed up during the 2nd year of life and group II during the 3rd year.

The following data are being collected :—

Weight, height, crown rump head circumference, chest circumference, abdominal circumference, biachromial diameter, bicristal diameter, girth of arm, girth of thigh, length of foot, length of hand, dentition, fontenel size and time of its closure.

156 (a) Studies on calcium, phosphorus and protein metabolism including utilization of mixtures of dietary vegetable proteins at the cutral Food Lecliro logical Research Institute, Mysorə.

The object of investigation:—

The objects of the investigation are as follows :

(i) To study the metabolism of calcium, phosphorus and protein in children and adult human subjects on diets based on cereals, and millets like rice, ragi, jowar, bajra and Italian millet.

(ii) To study the utilization of mixtures of dietary vegetable proteins by human beings

(iii) To study the role of cellulose and hemicellulose on the absorption of proteins, calcium and phosphorus.

Poor vegetarian diets based mainly on rice are deficient in the essential mineral, calcium. It is likely that the deficiency of calcium in the rice diets can be made up to some extent by replacing a part of the cereal by ragi. In view of this, investigations were undertaken to study the effect of replacing rice by ragi to varying extents in the poor rice diet on (i) the metabolism of nitrogen, calcium and phosphorus in children and (ii) on the growth and metabolism of nitrogen, calcium and phosphorus in rats.

Metabolism of nitrogen, calcium and phosphorus in children on diets based on rice and/or ragi.

The metabolism studies were carried on eight girls aged between 9 and 10 years. The metabolism experiments consisted of 4 periods. The proportion of rice to ragi fed during different periods were as follows : Period 1 rice 100 per cent ; Period 2. Rice 75 per cent + ragi 25 per cent ; Period 3, rice 50 per cent + ragi 50 per cent and Period 4, ragi 100 per cent. Other ingredients of the diet were the same in all the diets. The subjects were fed on each experimental diet for a period of 15 days, the first 10 days being treated as a preliminary period. The urine and faeces were collected for analysis during the last 5 days of each period and were analysed for N, Ca and P

Results : The mean daily excretion of N in the faeces increased as the level of ragi in the diet was raised and was the highest on the diet containing the entire cereal as ragi. The apparent digestibility coefficients of proteins from the different diets decreased as the ragi in the diet increased. All the subjects were in positive N balance on the different diets.

The replacement of rice by ragi in the diets of the children resulted in significant increase of the dietary calcium. The calcium balance on the three diets containing ragi was significantly higher than that on the rice diet.

All the subjects were in positive phosphorus balance on the different diets.

The results show that replacement of rice in poor rice diet by ragi to the extent of 25 per cent or 50 per cent brought about a significant increase in the retention of calcium and would be sufficient to make up the calcium deficiency in the poor rice diet.

Growth and matabolism studies on rats.

Growth and metabolism studies were carried out on rats fed on rice diet, or rice diet in which rice is replaced by ragi to the extent of 25 per cent, 50 per cent or 100 per cent. The results showed that substitution of rice in poor rice diet by ragi to the extent of 25 or 50 per cent produced a marked improvement in the nutritive value of the diet as judged by the growth of rats. The mean daily retention of N in the growing rats was higher on diets containing 25 per cent ragi than that observed on other diets. The mean daily retentions of calcium in the growing rats on diets containing 25 per cent, 50 per cent and 100 per cent ragi were significantly higher than that in rats fed on rice diet.

The mean daily retention of phosphorus in growing rats on poor rice diet was lower than that in rats fed on diets containing 25, 50 or 100 per cent of ragi. The results obtained in animal experiments also clearly show that replacement of rice in the diet by ragi even to the extent of 25 per cent will be adequate to make up the calcium deficiency in poor rice diets.

157 (b). "Nutritive value of Foodstuffs" Central Food Technological Research Institute, Mysore.

In continuation of the first phase of the programme of work namely filling up of gaps in materials listed in Health Bulletin No. 23, a further number of fifty five materials (cereals, pulses, oilseeds and nuts, condiments and vegetables), four different samples of each collected in Mysore, Bellary, Dharwar and Gulbarga, and in the case of perishable articles local samples only, have been analysed for vitamins, fibre and available phosphorus.

It is proposed to extend the work with respect to other materials listed in the above Bulletin during 1959-60 as per the agreed programme set by the working Party of the I.C.M.R. for this enquiry.

158. Investigations on field trials with protein rich foods under Dr. C. N. Rukmini at the Corporation of Madras, Madras.

The foods that are being tried under this scheme are: (1) Bengal gram and skim milk mixed with jaggery. (2) Skim milk—Reconstituted. (3) Mysore food A and (4) Mysore food B.

Bengal gram skim milk mixture.—The number of children enrolled for trials on this food were 82, out of which 37 discontinued during the course of the year leaving 45 on roll at the end of September, 1958.

Of the 45 children only 24 have been under observation for a year and more. The average daily attendance is satisfactory and the food is acceptable to all children, not one child having stopped away on account of non-acceptability.

Skim milk reconstituted.—The number of children enrolled were 76, out of which 70 discontinued during the year, 36 of them within a few days of enrolling due to initial diarrhoea. It is noticed that all the children invariably have diarrhoea for a few days at the beginning and that this diarrhoea stops of its own accord after a week. Unfortunately, the mothers who are willing to give the food a fair trial are very few. Only one child has been continuously under observation from July, 1957.

Mysore food A.—114 children were enrolled of which 85 discontinued during the year due to an epidemic of diarrhoea and dysentery in the locality, during July-September, 1957, may be taken to have accounted for a majority of them. The children are regular and eager for this food and there is no difficulty in their taking them daily full quota.

Mysore food B.—67 children were enrolled of which 57 discontinued by July, 1958. Even the 10 who were on roll had to be persuaded daily to go over to the Centre for the food, the average attendance being 4 or 5. Very rarely was the full quota of food taken by any child because of the slightly bitter taste. In view of these practical difficulties, trials on this food were discontinued from July, 1958.

159. Field trials with protein rich foods under Dr. Muktha Sen at the All-India Institute of Hygiene & Public Health, Calcutta.

The enquiry was started in October 1957 and the preliminary preparation in the form of base line survey continued till the end of March 1958. The children for the feeding and the control groups were statistically sorted out and the feeding was commenced in the last week of March 1958. In the early stages, however, several children were irregular because of gastro-intestinal infections in the hot months of April, May and June. The required numbers of 50 for each of the three groups, (1) feeding with Bengal gram (2) skimmed milk and (3) control, were available only from the end of August 1958. Since then the enquiry has been progressing satisfactorily. At present there are 59 children for the milk group, 57 for the vegetable protein (Bengal Gram) and 58 for the control.

The quantity of protein and the calories given to each age group are kept constant on both the diets. A detailed record of the growth and development and the incidence of sickness is maintained for all the subjects.

The age groups covered are 1-2, 2-3, 3-4, 4-5 and 5-6 years. Heights are taken every month and weights every 15 days. A nutritional assessment is carried out once in 3 months on all the children as far as possible. Attempts are also being made to estimate the total protein content in the blood which would be helpful evaluating the utility of the food that is being given to the child.

160. Clinical trials with protein-rich foods under Dr. R. N. Chaudhuri at the School of Tropical Medicine, Calcutta.

Fifty-six children, aged 1 to 5 years, suffering from protein malnutrition (marasmus 9 and kwashiorkor 47 cases) were treated in 3 groups with protein-rich vegetable foods prepared at the Central Food Technological Institute, Mysore; A is a mixture of defatted ground nut flour, roasted Bengal gram flour and lucerne powder and B that of defatted ground nut flour, roasted Bengal gram flour, sesame flour and lucerne powder. A B and skimmed milk powder. Each of these foods along with supplements used, provided 60 g. of protein and 1200 to 1500 calories per child.

The effects of a month's course of this diet-treatment were studied. In the cases selected oedema and diarrhoea were the commonest features. Next came emaciation, skin changes and vitamin deficiencies. In most of the cases the oedema subsided in 7 to 10 days and the diarrhoea stopped within a few days after admission. Nineteen out of 38 patients who completed one month's treatment gained 1 kg. in weight after reaching the minimum weight. With all the 3 foods there was rise of serum albumin, this being highest in children receiving skimmed milk.

It was observed that many children could not consume the prescribed amounts of vegetable foods especially in the earlier part of the trial. Some, even developed aversion for the food and had to be forced. In this way 38 children could complete the month's course; 4 were taken away from the hospital by their parents, 10 positively refused to take the food, the condition of 2 deteriorated and they died subsequently; one died of broncho-pneumonia on the 5th day. Two children developed obstinate skin changes which could not be cured. (It is not implied that this was the result of feeding).

However, in spite of these difficulties the vegetable foods to have a place in the treatment of protein malnutrition, but they are inferior to skimmed milk in respect of regeneration of Derum albumin.

It was suggested that the use of these foods be limited to treatment of comparatively milder cases and they may be of value for prevention of protein malnutrition.

Preparation of a gruel diet from rice, Bengal gram, milk and leafy vegetables and its clinical trial in Kwashiorkor :

A gruel diet from rice (2 parts), Bengal gram flour (3 parts) milk (8 ozs. a day) and leafy vegetables was prepared and its clinical trial was carried out in 8 cases of kwashiorkor. The food could be readily prepared at home, it was cheap, the taste was liked by the children and all the 6 cases, who completed one month's dietary treatment improved satisfactorily. Further trials are in progress.

161. Clinical trials with protein rich foods under Dr. S. T. Achar at the Madras Medical College and Government General Hospital, Madras.

Sixty-two children suffering from protein malnutrition were treated in three groups with protein-rich foods, Mysore A, Bengal gram,-skim milk mixture and skim milk powder.

Clinical features.—All had œdema ; next main features were diarrhœa, skin changes and hair changes in that order. Next came liver palpability, Vitamins A and B deficiencies

Oedema subsided in about 20 days' time and diarrhœa within a few days after admission in a majority of cases.

In the yearly stages children were refusing or even vomiting any of the three foods. In the improved stage children below 1½ years and above 4 years took any diet. Between 1½ and 4 years, there was a tendency to refuse Mysore food A and Bengal gram skim milk porridge. More cases of vomiting were also seen in those two groups. Diarrhoea was seen in some cases treated with skim milk.

Serum albumin rises quicker with the combination of vegetable and animal protein, next with pure animal protein and lastly with pure vegetable proteins.

162. Studies on human lactation under Dr. J. W. Airan at the Wilson College, Bombay.

Amino Acid composition of Human Milk.

This year the samples were collected as before during the period of lactation and analysed for their amino acid content by microbiological assay.

In all, 66 samples were analysed from 11 subjects. Of these, 6 came from low economic class, 4 from middle economic class, and only 1 from higher economic class. This classification into low, middle and higher economic classes was arbitrary.

The first sample in these subjects was collected on about the 10th day after delivery, as this could be easily done in the hospital. Subsequent samples were obtained at intervals of one week covering a total period of about one month after delivery. Further samples were collected at monthly intervals. Most of these cases are still being followed up.

The amino-acid content of the milk collected during the period of lactation, goes on decreasing, particularly from the third week onwards, except that phenylalanine in subjects from low and middle economic classes and isoleucine and leucine in subjects from middle and high economic classes show wide variations. In general, the pattern of amino acid composition during the period of lactation is more or less similar in the samples of milk of all the three groups studied so far.

163 Human milk studies under Dr. J. Ganguly at the Indian Institute of Science, Bangalore.

For a preliminary study of the essential amino acids arrangements were made to analyse some random samples of milk collected from local hospitals. The mothers remained in hospitals only for a few days after child-birth ; the samples collected during this period represented the "colostrum". With the co-operation of the R. M. O. of the Institute four donors who are rendering active co-operation in the present study were obtained.

Preliminary studies were carried out by circular paper chromatography method developed by Giri *et al.* Subsequently, some microbiological assays were also made.

Uptil now 50 samples of milk were analysed. These were collected from 5 mothers (follow-up cases) collected once a week over a period of lactation varying from 30 days to 150 days post partum. Two of the subjects were poor and non-vegetarian while the others were vegetarians from lower middle class.

The initial findings show that (i) the average protein content of the mature human milk in the period, 30 days to 100 days post partum is 1.0 g. per 100 ml. secretion, and (ii) the methionine and phenylalanine contents are low.

Further work on essential amino acids and B vitamin contents is in progress.

164. Studies on human lactation under Dr. C. V. Ramakrishnan at the Baroda University, Baroda.

Studies were made of the effects of socioeconomic status and stage of lactation on the chemical composition of human milk.

Two hundred milk samples from lactating women in different socio-economic groups (very poor, poor, middle and upper middle classes) and at different stages of lactation were analysed for (i) the proximate principles viz, total protein, lactalbumin plus lactoglobulin, casein, lactose, and fat, (ii) essential amino acids, (iii) vitamins B_1, B_2, and B_{12}, and C, pantothenic acid and nicotinic acid. Rapid diet survey on lactating women was also carried out.

The chemical composition of milk with respect to the above constituents was found not to vary significantly after the first month of lactation. Marked differences were not observed in the composition of milk of women belonging to different socio-economic groups. Nevertheless, some constituents were found to vary with socio-economic status. For instance, the protein and essential amino-acid content of milk was greater in the middle class than in the other groups studied. The fat content of milk in the very poor group was less than that in other groups.

In general, values for the protein content of milk in the subject studied were found to compare well with those reported for Australian and Bantu women and were somewhat higher than those reported for other women. The fat content also was found to compare well with that reported for Australian, British and American women while it was considerably higher than that reported for the others. On the other hand, values for vitamins were generally low as compared to other groups. The same was true with regard to cystine and methionine.

The results of the diet survey indicated that the diet of the lactating women in this region is generally inadequate in protein, calories and calcium.

165. Enquiry on protein metabolism in under-nourished and malnourished children under Dr. R.N. Chaudhuri at the School of Tropical Medicine, Calcutta.

One hundred and fifty under-nourished or malnourished children were studied. Of them 56 per cent were males and 44 per cent were females 78 per cent were between 6 months and 3 years of age. Most of them came from the poor income groups.

Seventy-six per cent of the children had severe emaciation with œdema (marasmic kwashiorkor—70 cases) or without œdema (marasmus-44 cases). The rest who were not so emaciated were almost equally divided between classical kwashiorkor (22 cases) and kwashiorkor without skin changes (16 cases).

Forty-three out of 150 mothers were either seriously ill (e.g. pulmonary tuberculosis) or grossly malnourished and 9 were dead. One-sixth the number of mothers had more than 5 children, while a few had more than 10 issues

Enquiry revealed ill-health or death among other children in their earlier years, in some families. On 22 occasions more than one child was admitted from the same family with similar clinical features.

About 16 per cent of the children never had breast-feeding owing to maternal causes. The remaining were breast-fed, 54 per cent from three months to one year and 24 per cent beyond this period About 28 per cent of the patients were being breast-fed at the time of admission. On two occasions mother's milk was measured, it was 10 to 12 oz. a day. The protein content, in 5 mothers, varied from 0.8 per cent to 1.2 per cent. Protein intake of these babies from the breast milk was 3 to 4 g. per day.

Supplementary feeds were started quite early in one-third of the children and by 6 months of age more than two thirds of the children were getting some form of supplements. The usual articles were cow's milk, barley, sago, dried tinned milk and in the case of older children rice, vegetables, bread, etc. Milk in any form was supplied in very small quantities. Protein content of the supplements was on an average about 10 g. Caloric intake was also very low. With the onset of diarrhœa all nourishment was cut down to barley, sago and candy water, usually according to the customs but not infrequently on medical advice.

The environment in which the patients lived and were fed, exposed them constantly to gastro-intestinal and other infections.

In a typical case, the mode of onset was as follows: A child was born either lean and thin or apparently healthy and was given sooner or later 4 to 8 oz. of cow's milk mixed with inadequate barley or sago water owing to scantiness of the breast milk. The child failed to grow, lost weight, got abdominal distension and on examination the child was a typical case of marasmus. If the diet was further curtailed at least temporarily due to diarrhœa, fever etc. he got œdema of legs and the resulting picture was marasmic kwashiorkor. Classical kwashiorkor and kwashiorkor without skin changes developed more often in children apparently wealthy but more or less suddenly exposed to conditions

in which the diet was reduced both in calories and protein. These conditions took a comparatively short time to develop in contrast to marasmus which took months to develop fully. A baby fed on a poor diet from the very start was more likely to develop marasmus than kwashiorkor.

Enlargement of liver was more commonly observed in cases of kwashiorkor. Dehydration as a result of diarrhœa was a very common feature. The diarrhœa was recurrent in marasmic cases. Fever (mostly due to pulmonary and other infections) and diarrhœa (due to dietetic errors or bacterial, protozoal or helminthic infections) being the two earliest symptoms, were suggestive of the contributory role of infections in the pathogenesis of these disorders.

Vitamins A and B deficiencies were common. rickets was rare and vitamin C deficiency was not met with. Mental changes, skin and hair changes, moon-face etc. were also observed.

Examination of stools revealed low incidence of helminthic (about 10 per cent), and other parasitic infestations. Microscopically pus cells were frequently observed but bacterial culture failed to reveal any organism except *E. coli*. Urine examination revealed a trace of albumin at times.

X-ray showed tuberculous infiltration of the lungs in 25, and pleural or lymph node involvement in 8 more cases. The incidence was greater in marasmic kwashiorkor. Linear lines, suggestive of arrest of bone growth at the lower end of the radius, were demonstrated in one third of the children so examined.

Blood examination showed moderate anæmia, Serum proteins were below normal. Anæmia and hypoproteinæmia were comparatively less severe in marasmus.

Nitrogen balance studies were done in 26 cases, the desired amount of protein being supplied from skimmed milk. Results varied considerably in individual cases and absorption was not usually affected in the absence of diarrhœa. In cases of nutritional marasmus at 60 g. of protein intake, per day a good part of the nitrogen absorbed was excreted in urine although there was still high nitrogen retention. Nitrogen retention continued to be equally high even after a month's diet-treatment. At 40 g. of protein intake nitrogen retention was comparatively lower. In classical kwashiorkor the retention was much higher than in marasmus, suggesting that the protein deficiency was more acute in the former. Cases of marasmus kwashiorkor showed the nitrogen retention pattern of marasmus and kwashiorkor in combination. While before treatment nitrogen retention was higher, after initial treatment they behaved as cases of marasmus. Nothing definite could be ascertained about the cases of kwaskiorhor without skin changes. Nitrogen retention was low in one such case and was further lowered after treatment. In cases having mild infestation with ascariasis absorption was slightly affected. Children infected with pulmonary tuberculosis could retain a good amount of nitrogen although no specific treatment was given.

Skimmed milk formed the sheet anchor in the treatment of these cases. To start with, small frequent feeds and even tube-feeding if

necessary were highly useful. Results of treatment unless the cases were too advanced or complicated were on the whole satisfactory. Clinical and biochemical improvement in marasmus varied from that observed in kwashiorkor. There were 39 deaths; 34 died before any effective treatment could be given and 5 died due to other infective diseases after treatment of the nutritional disorder. Of the important causes of death, diarrhœa was responsible in 19 children, bronchopneumonia in 4 and pulmonary tuberculosis in 2. However, in 8 cases death was quite sudden and could not be explained from the clinical findings.

Post mortem could be done in 20 cases. Important findings were fatty liver, pneumonia, bronchopneumonia, empyema, pulmonary and intestinal tuberculosis, bilateral adrenal hæmorrhage, œsophageal ulcers, congested and/or ulcerated and distended intestines etc. It appeared that the extent of the fatty liver was directly proportional to the amount of subcutaneous and other depot fat.

166. Enquiry into the protein requirements in pregnancy and lactation under Dr. H. N. Banerji at the G. R. Medical College, Gwalior.

During the current year, 18 healthy pregnant women were included in the study, bringing the total to 26 subjects. Of the 18 pregnant subjects, 6 were in the scond trimester and 12 in the third trimester. All the subjects were considered clinically normal after a check-up of blood pressure, routine urine examination and blood picture. Some of the baseline features in these cases were :—

Age—Varied from 18 years to 42 years.

Parity—Varied from primipara to ninth para.

Height and Weight—55″—61.5″ ; 34.9 kg. to 62.6 kg.

Biochemical Assessment— Hb.—76 per cent to 101 per cent, plasma proteins varied from 6.25 to 7.3g. per cent.

Dietary Habits—All were vegetarians consuming about 1600-1900 calories with about 40-50 g. of proteins per day. Milk proteins contributed to 15-20 per cent of the total proteins. Ca, P, Fe, and Vitamins were less than the recommended allowances in their home diets.

During the experimental period the subjects were put on diets with varying levels of proteins and calories. These diets were adequate with reference to other nutrients In general about 60 g. of protein were provided at a caloric intake level of 2300 to 2400 and about 90 to 100 g of protein with 2400 to 2600 calories : Milk proteins formed 20 to 60 pc. of the total dietary proteins. Subjects were stabilized on the diets for 4 7 days and on the following three consecutive days entire urine and fæces were collected over toluene for total nitrogen estimations. Creatinine in urine was estimated as a check on the output in every case.

Three subjects were studied in second trimester of pregnancy on 60 g. and 100 g. levels of protein in continuity. One was studied on 90 and 120 g. levels of intake.

In the third trimester of pregnancy, two were studied on 60 and 90 g. levels of protein intake, one on 90, 100 and 81 g. levels of protein intake, and two on 60 and 100 g. levels of protein intake.

The results showed that 60 g. level of protein intake with 2400-2500 C. generally led to a negative nitrogen balance, though some were in the neighbourhood of nitrogen equallibeium. With 90 and 100 g. of proteisn and 2400-2600 calories, positive nitrogen balance was achieved. In this range of protein intake, the proteins contributed more than 14 per cent of thetotal calories.

It is inferred that the protein requirement of healthy pregnant Indian women subsisting on vegetarian diets with body weights ranging between 40 kg. to 63 kg. would be about 85 g. with about 2600 calories. This is an agreement with the recommendations of Food and Nutrition Board of National Research Council.

167. Enquiry on protein requirements in pregnancy under Dr. M.P. John at the P.W. Medical College, Patna.

This work was undertaken to find out the minimum amounts of protein necessary at different periods of pregnancy. So far the investigations were completed on 15 women in different periods of pregnancy.

The women were given diets containing three different levels of protein, viz., 60 to 70 g. 90 g. and 120 g per day. Total caloric intake was kept under 3000 in 24 hours. In some cases, the diet contained both animal and vegetable proteins in and equal proportions. It was, however, found difficult to give the vegetarians diet containing more than 110 g. of protein per day without causing digestive upset. An accurate assessment of the nitrogen intake was made by analysing the dietary ingredients individually. If any part of the diet was left unconsumed, its nitrogen content was estimated and appropriate corrections applied to the intake figures. A constant watch was kept to see that the subjects under investigation do not take any food other than the prescribed diet.

24-hour collections were made both for urine and faeces. Faeces was collected by using charcoal tablets as markers. The samples were preserved in ice chest and N. estimations were carried out in duplicate within 24 hours after collection.

OBSERVATIONS

Nitrogen retention was found to vary from case to case. As the amount of nitrogen was increased in the diet its retention was also increased but the proportion of nitrogen retained did not run parallel with the increase in the dietary nitrogen.

In three cases the nitrogen retention was investigated serially at different periods during pregnancy; these cases showed no definite increase in the amount of nitrogen retained as pregnancy advanced.

A high protein diet did appear to prevent premature onset of labour. Thirteen cases with previous history of premature labour, where no cause could be found, were given high protein diet and twelve of them went to full-term and delivered normally.

168. Studies on energy metabolism under Dr. Shiv Kumar at the Medical College, Amritsar.

Basal metabolic rate was determined by respirometric method in 100 apparently normal healthy male subjects drawn from the local police force. Using a Douglas bag, a Haldane-Henderson-Bailey gas analysis apparatus and a K. M. Respirometer, ventilation, oxygen consumption and CO_2 production per minute were determined. The respiratory quotient was calculated and B.M.R. determined. The results were expressed as calories per square meter per hour. Two determinations were made on each subject. Age, weight, and height were recorded in each case. The relevant data are tabulated below :—

TABLE

Serial No.	Age group.	No. of subjects	Mean B.M.R. (calories per sq. meter)	Standard deviation.	Standard error of mean.
1.	20-29 years	38	35.10	± 1.2305	± 0.1411
2.	30-39 ,,	36	34.08	± 1.5902	± 0.1874
3.	40-49 ,,	17	33.72	± 1.6395	± 0.2812
4.	50-55 ,,	9	31.90	± 0.6768	± 0.1595

It was observed that the basal metabolic rate declined with age, that the mean B.M.R. values were on an average 14·35 pc below the Mayo Foundation standards, and that in a vast majority of the subjects the B.M.R. bore a linear relationship to weight.

Further work is in progress with a view to assess the effect, if any, of seasonal variations on B.M.R.

169. Studies on energy metabolism under Dr. S. Banerjee at the Presidency College, Calcutta.

I. *Determination of the Surface Area of the Body of Indian Females.*

As the physique of women differs to a great extent from that of men, the surface area in women was actually measured to find out if the latter differed from that calculated from the formula of DuBois and DuBois or of Banerjee and Sen.

Surface areas of the bodies of seven adult women were actually measured using a combination of tape, surface integrator, and mould methods. Body surface areas actually measured, were greater than those calculated from the weight height formula of DuBois and DuBois. Based on the measurement, a new constant for the weight height formula of DuBois and DuBois has been suggested for the determination of the surface area of the body of women. The suggested equation runs as :—

$$\underset{cm^2}{\text{Surface Area}} \quad \underset{(kg.)}{\text{Wt. } 0.425} \quad \times \underset{(cm.)}{\text{Ht. } 0\ 725} \quad \times 78.28$$

A nomogram based on the above formula has been prepared.

The body surface area was also calculated using the linear formula of DuBois and DuBois but the results obtained were at variance with the area actually measured. New constants have been suggested for determining the surface area of various parts of the body using linear formula. It has been suggested that weight height equation with the new constant should be preferred to the linear formula.

II. *Determination of Basal Metabolic Rate and Blood Concentration of Protein Bound Iodine, Cholesterol and Glucose in Indians.*

To account for the low B.M.R. among Indians it is considered desirable to find out if the former suffer from slight hypofunction of the thyroid gland not associated with any clinical symptoms. In order to assess the functional activity of the thyroid gland. Serum protein bound iodine (P.B.I.), serum cholesterol and fasting blood sugar were determined in subjects whose B.M R. was studied.

B.M.R. measured by the open circuit method was on the average 12 pc. lower than the Mayo Foundation Standards in the case of females, and 17 pc. lower in the case of males. The close circuit method usually gave still lower value than the open method. Serum protein bound iodine, serum cholesterol and blood sugar of the subjects were found to lie within the normal ranges, indicating no abnormality in the thyroid function. The low B.M.R. of Indians may perhaps be due to the reduced amount of metabolically active component in the body.

Studies on Energy Expenditure in Laboratory Workers.

The energy expenditure during different activities was determined in a group of laboratory workers, by the measurement of oxygen consumption using Maxplanck Institute respirometer and Haldane-Henderson-Bailey

gas analysis apparatus. The energy cost of various activities expressed as calories per square meter body surface per hour is as follows :—

B.M.R. 30.87 ± 0.741 ; lying in rest 34.79 ± 0.578 ; sitting at study 42.85 ± 1.034 ; sitting at work 46.79 ± 3.036 ; standing at laboratory work 59.96 ± 3.937 ; standing at household work 61.78 ± 3.675 ; descending stairs 95.80 ± 6.30 ; ascending stairs 159.94 ± 5.610 ; and walking 122.66 ± 6.25. The total daily energy expenditure of the subjects determined by diary technique of Garry *et al.* over a period of 7 days varied between 1620 and 2927 calories per day.

The average food actually consumed by the subjects was analysed for fat, protein and carbohydrate to determine the caloric value. The intake varied between 1680 and 2928 calories per day.

170. Metabolic studies in the B group of vitamins with special reference to folic acid and vitamin B_{12} under Dr. A. Sreenivasan at the University of Bombay, Bombay.

1. *Glycine-Choline Interconversions.*

Evidence for the involvement of folic acid in glycine to choline and serine to choline conversions in rats was presented in the previous report. The observed decrease *in vivo* in hippuric acid synthesis in folic acid deficiency was explained as probably due to decreased availability, in the absence of folic acid, of endogenous free (non-protein) glycine, which is only utilized for conjugation (*Biochem. J.*, 65, 580,1957) either from serine (*Biochem J.*, 55, 278, 1953) or from choline (*J. Biol. Chem.*, 204, 207, 1953)

Conversion of choline to free glycine and free serine in relation to folic acid has been studied presently in normal and benzoate treated rats. In conditions of induced free glycine depletion by benzoate administration, choline produces marked reversal of free glycine levels, the effect being comparable to that of folic acid. Choline and folic acid together, however, do not bring about any further increase. Benzoate administration also causes a notable increase in liver non-protein nitrogen, the increase being more significant in folic acid or choline fed groups. Free serine levels do not show appreciable alterations under above conditions.

2. *Glycine Biosynthesis*

Although it is generally considered that serine is quantitatively by far the most important immediate precursor of glycine, the relative effectiveness of other compounds like choline as precursors, and the specific functions of vitamins like folic acid and vitamin B_{12} in the biosynthetic processes do not appear to have been adequately investigated. A re-evaluation of the present ideas on glycine biosynthesis and glycine-serine interconversions has also been necessitated in view of certain observations reported recently (*Biochem J.*, 67, 287, 1957).

It is observed that there is a significont decrease in free glycine levels of blood and liver of rats in folic acid deficiency, and that benzoate administration causes a marked further lowering. Free serine levels of blood and liver, however, do not show significant alterations. Forced *in vivo* conversions of S→G or G→S appear to take place freely with the mediation of folic acid. It is suggested therefore that even though reversible interconversions of glycine and serine could be suitably brought about either *in vitro* or under forced conditions *in vivo*, the reaction S→G appears to be of major physiological significance in rats, its extent being determined by the availability of folic acid. This view is also supported by observations that benzoate administration and subsequent free glycine depletion is readily followed by decrease in free serine levels in the early period, the levels being steadily restored to normal later. Further work is in progress to study these and other aspects of glycine biosynthesis in more detail.

3. *Vitamin B_{12} and Oxidative Metabolism*

Studies have been reported previously on protective action of vitamin B_{12} against nutritional stress conditions of thyrotoxicosis and carbon

tetrachloride liver injury in rats. The work was continued further on the following two lines (a) Magnesium metabolism in thyrotoxicosis and the effect of vitamin B_{12}, and (b) Sulphydryl metabolism.

(a) *Magnesium metabolism*

A close relationship of thyroxine to magnesium metabolism has been indicated in various studies on oxidative phosphorylation and recently it has been shown that growth retardation and uncoupling of oxidative phosphorylation caused by thyroxine administration is counteracted by extra supplementation of dietary magnesium (*J. Biol Chem.*, 226, 597, 1957). In the experiments reported here, it is seen that a marked reduction in plasma levels of free magnesium occurs in thyrotoxicosis, and that vitamin B_{12} supplementation brings about a significant improvement. Reversal of decreased magnesium levels in isolated mitochondria from thyrotoxic rats by vitamin B_{12} has also been reported from this laboratory.

(b) *Sulphydryl metabolism*

The incorporation of administered cysteine into liver SH, reported previously, has been found partially in nuclear glutathione. However, liver perfusion with an equimolar mixture of the constituent amino acids did not result in a significant rise in the nuclear glutathione. Also attempts to demonstrate synthesis of glutathione *in vitro* with rat liver nuclei were unsuccessful probably due to the presence of active cysteine desulphydrase.

The existence in rat blood erythrocytes of an enzyme system capable of conjugating the constituent amino acids to form glutathione has been shown. The system appears to require Mg^{++} and K^{+} ions and is stimulated by adenosine or AMP. The effects of dietary folic acid and vitamin B_{12} deficiencies on this *in vitro* synthesis of glutathione has been studied. Folic acid has little effect while vitamin B_{12} deficiency causes a definite impairment in glutathione synthesis by erythrocytes.

4. *Nitrogen retention in relation to dietary fat levels*

Proper utilization of proteins requires adequate amount of calories. and substitution of carbohydrates by fat is known to result in decreased nitrogen retention (*J. Nutrition*, 56, 139, 1955). Such dietary alterations are expected to modify requirements of B vitamins, and particularly on high fat diets increased requirement of lipotropic factors is anticipated.

A favourable effect of folic acid on nitrogen retention in rats fed a peanut protein diet (18pc protein) with 4 and 10 pc supplements of oil, was reported previously. In continuation of these studies, effects of folic acid and vitamin B_{12}, either alone or together, were studied on a 10 p.c. casein diet with 4 and 10 pc. fat levels. It is evident from the results of this experiment that folic acid, but not vitamin B_{12} is effective in partially correcting the nitrogen imbalance caused by high dietary fat. However, the two vitamins together bring about a near-complete correction. The effectiveness of a mixture of choline and methionine (0.3 pc. each) in overcoming the nitrogen imbalance on a high fat (15 pc.) 18 pc. casein diet is found to be comparable to that of folic acid alone.

5. *Methionine oxidation.*

Although it has been shown previously that folic acid influences transmethylations favourably by virtue of its controlling influence of formate utilization in single carbon addition reactions, thus resulting in "a bleeding off" of the methyl compounds into formate in folic acid deficiency, a direct proof that in folic acid deficiency methionine oxidation to formate increases, seemed necessary.

The decrease in *in vitro* methionine oxidation by liver preparations of rats reared on a low-choline low-methionine diet in folic acid deficiency was explained earlier as due probably to a quantitative difference in the effects of the vitamin on (i) methionine to formate conversion, and (ii) formate oxidation to carbon dioxide. This observation was extended to a study of the effect of dietary methionine and folic acid on urinary excretion of formate in rats, which was found to be markedly increased in folic acid deficiency.

Results of *in vitro* experiments to follow the formation of labelled formate and carbon dioxide from methionine-$C^{14}H_3$ in liver homogenates also suggest an increased oxidative degradation of methionine to formate in folic acid deficiency (*J. Biol. Chem.*, 233, 252, 1958). The difference in *in vitro* formation of carbon dioxide in the deficient and replete groups, however, is not significant.

6. *Vitamin B_{12} binding by rat serum protein fractions.*

Studies on the binding of vitamin B_{12} by rat serum protein fractions were completed. In normal serum the vitamin was found to exist largely in the bound form, almost entirely associated with the a^2 globulin fraction. Following graded additions of the vitamin to serum, up to 1.5 mug/ml. the vitamin was mainly recovered in the a^2-and β-globulin fractions, the bound vitamin being confined to the a^2-fraction. With further additions, the vitamin migrated in the free state with the 8-globulin fraction. These observations have been extended to present evidence for a possible involvement of the serum β- globulin fraction in the transportation of vitamin B_{12} to its functional sites (*Ind. Jour Med. Res.*, 46, 690, 1958).

7. *Hemoglobin synthesis*

The conditions influencing optimal *in vitro* synthesis of hemoglobin by (i) rat blood reticulocytes and (ii) immature blood cells of vitamin B_{12} and folic acid deficient rats, from essential amino acids, were examined. Among optimal conditions established for reticulocytes were the integrity of the cells and aerobic conditions of the medium. Additions of glycine, iron, and plasma from phenylhydrazine treated rats stimulated the synthesis. An observed inhibitory effect due to plasma from normal animals could be reversed by the prior saturation of plasma with iron (Fe^{++}) or by heat denaturation of its proteins.

Reticulocytes were capable of synthesizing considerable hemoglobin in plasma from vitamin B_{12} and folic acid deficient animals, which effect was attributable to the adequacy of essential amino acids in the plasma. Blood cells of the deficient animals, in contrast to reticulocytes, failed to synthesize significant quantities of hemoglobin from essential amino acids and glycine. Administration of the vitamins to the

deficient animals 16 hours prior to the experiment did not improve the hemoglobin synthesizing ability of the cells unless glycine was also added to the reaction mixture.

8. *Phenylhydrazine hemolysis and hemoglobin degradation.*

The foregoing studies on hemoglobin synthesis have also indicated that the decrease in blood hemoglobin and increase in reticulocyte count and spleen size during phenylhydrazine treatment of rats, are attended by marked lowering of serum vitamin B_{12} level and gross alterations in the electrophoretic pattern of serum proteins. Many of these changes could be attributed to a decreased hepatic function, excessive destruction of erythrocytes and, possibly, plasma cells, and increase in the degradative products of hemoglobin, viz. bilirubin and iron, in the serum.

Evidence was obtained for the presence in normal plasma of a heat stable factor causing release of hemoglobin from damaged erythrocytes. During incubation of spleen with plasma and red cell stroma the hemoglobin released from the latter underwent modifications as indicated by a shift of the Soret band and excessive deposition of iron in the spleen. The observations point to a possible involvement of the spleen in the degradation of hemoglobin.

171. Studies on metabolism of vitamin K and riboflavin under Dr. S. C. Roy at the University College of Science and Technology, Calcutta.

1. (*a*) *Purification of prothrombin and determination of its chemical composition.*

Highly purified prothrombin was prepared from ox-blood by the technique already described in last year's report. The preparations had an average activity of 1600-1700 units per mg. The elementary composition on the ash-free-moisture-free basis is as follows :—N 14.6 pc. H 6.77pc. C-46.11 pc. and S-1.1 pc. The product has a moisture content of 8.15pc. and ash content 2.8 pc. The tyrosine content is 4.38 pc. as determined by the method of Udenfriend and Cooper (J. Biol. Chem., 196, 227, 1952). The tryptophan content of prothrombin is 3.4 pc as determined by the method of Graham *et al.*(J. Biol. Chem., 168, 711, 1947). The cystine content determined by the method of Sullivan and Hess (J. Biol. Chem., 117, 243, 1937) is 3.1› pc. The rest of the amino acids are determined by the (quantitative paper chromatographic technique. The results are as follows in per cent) : Lysine—5.75, Aspartic—13.4, Glutamic—13.3, Threonine—4,65 Alanine—4.1, Methionine—2.06, Valine—4.23, Phenylalanine—4,33 Leucines—10.4 and Proline—5.11.

Prothrombin is known to be a glycoprotein (Seegers *et al.*, Aroh. Biochem. and Biophys., 49, 276, 1954), and its reducing sugar content is determined by the method of Park and Johnson (J. Biol. Chem., 181, 149, 1949) andfound to be 6.5 pc. as expressed in terms of glucose. The pentose content as determined in terms of ribose is 0.61 pc. (Mejbaum, Hoppe-Sepler's Z. Physiol. Chem., 258, 117, 1939).

(*b*) *Further fractionation and characterization of the oily product obtaied from prothrombin by treatment with Weber's solution.*

The prothrombin preparation (1600 u/mg.) on treatment with Weber's solution yielded an oily fraction with vitamin K-like activity which was further fractionated as described below :

The crude sample, which consisted of oil sparsely dispersed with some solid material, was clarfied by treatment with charcoal and a column of $ZnCO_3$ and celite (3 : 1). The oily stuff was then partitioned out in solvents like cardondisulphide, acetone, methyl alcohol and petroleum ether.

The infra-red spectra of the different fractions were obtained and the results were compared with the infra-red spectra of dihydro-K_1-diacetate and 2-methyl, 1,4-naphthoquinone. The following characteristic groups are present, *viz.* (1) -OH group, (2) unconjugated =C=O, not ester like acetate, fomate, etc. and (3) a side methyl group in all the test and standard compounds. The absorption maxima in the region of 6.1 μ characteristic of dihydro-vitamin K_1-diacetate and 6.01, 6.18 and 6.23 μ characteristic of 2-methyl-1 : 4-naphthoquinone, are absent in the test compounds obtained from prothrombin. So the relation between naphthoquinone structure and vitamin K activity does not seem to hold true for the oil fractions obtained from prothrombin. Biological experi-

ments are in progress for a clear resolution of this discrepancy arising out of the infra-red studies.

(c) *Effect of different metallic ions on the activation of prothrombin:*

In order to see the effect of different metallic ions on the activation of prothrombin the following metals were tried :—Ca, Zn, Co, Ni, Fe, Cu and Cd. Thrombin formation was measured by the two-stage procedure of Ware and Seegers (Am. J. Clin. Pathol., 19, 471, 1949). It was found that while Fe^{+++}, Cu^{++}, Zn^{++} completely inhibit the thrombin formation, Co^{++}, Ni^{++} and Cd^{++} activate its formation like Ca^{++}.

II. (a) Studies on riboflavin :

To study the metabolism of the bacterium *Pseudomonas sp.* for the synthesis and the optimal yield of riboflavin and its analogue both the synthetic and the complex media were tried. With medium consisting of glycerol, NaCl, K_2HPO_4, $(NH_4)_2HPO_4$, $MgSO_4$ at a pH 6.8, a blue fluorrescent pigment was produced with relatively very small yield of riboflavin.

With complex medium consisting of 1 pc. casein hydrolyzate at pH 6.8 to 7 at 37°C. yield of riboflavin was higher with a small amount of the blue fluorescent compound.

(b) For the isolation, purification and final crystallization of the new analogue already identified, various solvents such as *n*-butanol, *n*-amyl alcohol, amyl acetate, ethyl acetate, phenol, benzyl alcohol and pyridine were tried alone or in combination. Extraction with phenol was found to be efficient under normal conditions, though extraction with pyridine gave higher yield. Its miscibility with water being very high at ordinary temperature, phenol was ultimately chosen. The phenol was removed from the extract by ether and the compound was freed from riboflavin by extraction with butanol and precipitated with absolute alcohol, and the product was isolated as yellow powder (yield 200 μg/ml.). This was subjected to further purification by column chromatography.

172. Studies on carbohydrate, vitamin and mineral metabolism under Dr. M. C. Nath at the Nagpur University, Nagpur.

(I) *Isolation and identification of 1:2 dienol glucose in the ccid hydrolysate of Glucose-cyclo-acetoacetate.*

Glucose-cyclo-acetoacetate (GCA) was hydrolysed for 20 minutes over a boiling water-bath with 2N HCl. On cooling, it was neutralised to pH 7 and extracted thrice with ether. A portion of the aqueous extract was spotted on the six circular discs of 18.5 cm. diameter from Whatmann Filter Paper No. 1. The discs were lightly pressed between two thick square glass plates and uniformly irrigated by butanol-acetic-acid-water (5:1:4) mixture. The position of the enolic compound was located by developing blue colour with 10 p c. phosphomolybdic acid in butanol. It was then eluted with hot water and dried at room temperature. The Dry substance also gave a positive test for an enol compound.

Another portion of the aqueous extract of GCA hydrolysate on distilling with 1 ON NaOH gave formaldehyde and a pontose which was identified to be arabinose. The breakdown of an enolic sugar to arabinose and formaldehyde shows its structure to be 1:2 dienol glucose.

(II) *Cure of alloxan diabetes by combined therapy with glucose-cyclo-acetoacetate and oestrogen.*

Seven groups of rabbits were taken and made diabetic with alloxan monohydrate. Oestrogen, sodium salt of GCA and aqueous extract of GCA hydrolysate were administered. The following observations wer found to be of interest.

(1) A does of 600 mg. kg. dody wt, of GCA or its hydrolysate was found to be more effective than a does of 300 mg. kg. body wt.

(2) Neither GCA nor oestrogen singly cured the diabetes completely.

(3) GCA coupled with oestrogen cured 4 out of 9 rabbits in seven weeks, but aqueous extract of GCA hydrolysate and oestrogen together were able to cure 5 out of 9 animals in five weeks showing thereby the greater activity of extract of, over GCA.

(4) Animals cured by oestrogen and GCA hydrolysate not only recoverd the initial loss in their weights, but also gained extra weight.

(III) *Studies on the utility of aqueous extract from GCA hydrolysale as compared with vitamin B_{12} in checking the depletion of reduced glutathione in the liver of rats fed low protein,-SH and vitamin E deficient diet.*

Different groups of rats were fed on SH and vitamin E deficient diet. Aqueous extract of GCA hydrolysate and vitamin B_{12} were injected subcutaneously. Content of reduced glutathione (GSH) in liver was estimated, It was interesting to observe that aqueous extract of GAC hydrolysate checked the depletion of GSH to the same extent as vitamin B_{12} did.

173. Studies in carbohydrate, fat vitamin and mineral metabolism under Dr. P.S. Sarma at the University of Madras, Madras.

Metabolism of Nicotinic acid and Nicotinamide :

Studies on the metabolism of nicotinic acid and nicotinamide have revealed that the deamidation of nicotinamide to nicotinic acid and ammonia is the principal feature of the metabolism of the vitamin in the mould *Neurospora* and in the insect *Corcyra cephalonica St.* Cell-free extracts of the tissues of these organisms, as also of the tissues of the pigeon and the chick, have been found to carry out deamidation of nicotinamide *in vitro.* The properties of the concerned enzyme in various tissues have been studied. The enzyme is associated exclusively with the soluble cytoplasmic material in cells from *Neurospora* and *Corcyra* tissue, while almost all the activity in the pigeon liver and kidney is found to be present in the particulate cellular components.

The deamidase enzyme in *N. Crassa* and in *Corcyra* has pH optimum between 6.5 and 8.0. The metal chelating agents $\alpha-\alpha-$Dipyridyl, 8-hydroxyquinoline and versene have been found to inhibit the deamidation of nicotinamide. In studies on the enzyme from *Corcyra*, the inhibition of enzyme activity by dipyridyl has been found to be overcome by Fe^{++}.

The use of C^{14} labelled nicotinic acid and nicotinamide has shown that the only excretory products on feeding either form of the vitamin to *Corcyra* larvae, are free nicotinic acid and nicotinuric acid. The use of C^{14} nicotinic acid has also been made in studying the *in vitro* conjugation of nicotinic acid with glycine by cell-free extracts of *Corcyra* larval tissue and by extracts of pigeon liver and kidney. The larval extract was found to carry out the *in vitro* conjugation, as indicated by the presence of a spot corresponding to nicotinuric acid in radioautograms of the incubation mixture. In the absence of an energy source, the synthesis does not take place. Similar *in vitro* synthesis has been found to be effected by pigeon kidney.

A rapid paper electrophoretic method for the separation of nicotinic acid, nicotinamide and derivatives has been developed. Veronal buffer and borate buffer, both at pH 8.6, have been found to give excellent separation of the various derivatives.

Lipid Metabolism and Orotic acid :

Orotic acid has been found to inhibit the growth of the larvæ of *Corcyra cephalonica St.* Among a number of compounds tried, methionine and choline have been found to reverse the growth inhibition. The tissue choline content of the larvæ fed orotic acid has been found to be about 45 p.c. more than that of larvæ fed a normal diet. The complicated relationship between orotic acid and choline indicates a possible effect of orotic acid on phospholipid metabolism. The effect of orotic acid on phospholipid synthesis and on incorporation of choline into phospholipids is under investigation.

An experiment was conducted to study the effect of carbon tetrachloride toxicity on the liver orotic acid content of rats fed a poor rice diet. Estimation of the orotic acid contents of the livers of normal and CCl_4-treated rats showed a significant increase in the orotic acid levels of the livers of the treated rats.

174. Studies on carbohydrate, vitamin and mineral metabolism under Dr. S. Banerjee at the Presidency Cosllege, Calcutta.

Studies on Protein and Carbohydrate Metabolism as Affected by Vitamin B_{12} and Folic Acid Deficiencies in Rats.

Combined deficiency of vitamin B_{12} and folic acid was produced in weanling rats by feeding a diet deficient in the above vitamins, and containing iodinated casein and sulphasuxidine. The urinary excretion of different nitrogenous conscituents such as non-protein nitrogen, urea nitrogen, uric acid, amino acid nitrogen, creatinine and creatine, was estimated during the progress of the combined vitamin deficiencies. All the nitrogenous constituents except creatine were excreted in increased amounts in vitamin B_{12} and folic acid deficient animals.

Glucose, total protein and non-protein nitrogen contents of blood, and total protein content of liver were determined in normal an deficient animals. No change in protein content of blood and liver could be obtained in deficient animals. The non-protein nitrogen of blood was found to be high, and the fasting blood sugar value low in deficient animals.

Glucose content of blood 2½ hours after oral administration of glucose was determined in normal and deficient animals. While in normal animals this blood sugar level was similar to the fasting value, the same was significantly higher in deficient animals.

Glycogen content of liver and muscle was determined before and 2½ hours after the oral administration of glucoso in the normal as well as deficient animals. The deficient animals showed a very low glycogen content in liver which did not increase after the administration of glucose. The muscle glycogen of deficient animals was unaffected and did not change after the administration of glucose.

Persistent increased urinary excretion of non-protein nitrogen, (urea, ammonia, amino acid, creatinine and uric acid) in vitamin B_{12} and folic acid deficient rats suggests that these vitamins are concerned in protein metabolism. It has been observed that in rats suffering from combined deficiency of these vitamins the utilization of glucoso was defective. In the deficiency state the glycogen value of the liver decreased significantly. These vitamins, therefore, are concerned with the deposition of glycogen in the liver.

Histochemical and Histological Studies in Normal and Folic Acid and vitamin B_{12} Deficient Rats.

The distribution of alkaline and acid phosphatases, ribonucleic acid (R.N.A.), and desoxyribonucleic acid (D.N.A.) was studied by histochemical technique in normal, and vitamin B_{12} and folic acid deficient rats. Tissues were also stained with haematoxylin and eosin for histological study.

The alkaline phosphatase was increased in kidney, adrenals, spleen, testes and thyroid in all animals made deficient in the two vitamins. In the deficient animals the acid phosphatase activity increased in liver and adrenals, and disappeared completely in the pituitary.

The vitamin B_{12} and folic acid deficient rats showed diminished R N.A. content in liver, pancreas, adrenals, spleen and testes, diminished D.N.A. content in pancreas, adrenals and testes, and increased DNA content in spleen. The deficient animals showed marked histologica changes in liver, thyroid, testes and spleen.

Phosphatases play an important role in the metabolism of carbohy drates, lipids and nucleoproteins. The changes observed in alkaline and acid phosphatase contents of tissues in vitamin B_{12} and folic acid deficiency may add to the evidence of the role of these two vitamins in carbohydrate metabolism.

The considerable increase observed in the alkaline phosphatase content of the adrenals of deficient rats may indicate a disturbed physio logical function of the gland.

D.N.A.and R.N.A. are found to be decreased in most of the tissue of the vitamin B_{12} and folic acid deficient rats which indicates the rela tions of these vitamins in nucleoprotein metabolism.

Nicotinic Acid-Tryptophan Metabolism in Man.

Twenty-four-hour urinary excretions of nicotinic acid and amide quinolinic acid, N'-methylnicotinamide, 6-pyridone, tryptophan kynurenin, anthranilic acid and 3-hydroxy-anthranilic acid were deter mined in 10 adult men both before and after the feeding of 5 g. of DL tryptophan on two consecutive days.

Normally urine contained nicotinic acid and amide, quinolini acid, N'—methylnicotinamide, 6-pyridone and tryptophan, and excre tion of these metabolites was increased after the feeding of tryptophan The increase was maximum in the excretion of 6-pyridone.

Kynurenin and 3-hydroxyanthranilic acid which were originall absent in urine appeared after the feeding of tryptophan. Anthranili acid could not be estimated in the urine even after the administration o tryptophan.

Protein Metabolism in Scorbutic Monkeys.

Blood and urinary excretion levels of non-protein nitrogen, (ure nitrogen, amino acid, nitrogen and creatinine, and urinary excretion o ammonia nitrogen, uric acid and creatinine) were determined in rhesu monkeys during the development of scurvy.

After the withdrawal of ascorbic acid from the diet for 3½ months when the animals had become severely scorbutic, blood levels of non protein nitrogen, urea nitrogen and creatinine increased and increased amounts of non-protein nitrogen, urea nitrogen and amino acid nitrogen were eliminated in the urine. Creatine was absent in normal urine bu appeard when animals developed scurvy.

Protein content of liver of scorbutic monkeys was less than that in normal monkeys.

Urinary excretion of total tyrosine derivatives, keto acids, homogentisic acid, tyrosine and phenylalanine was estimated in rhesus monkeys, during the development of scurvy, both before and after a load dose of tyrosine and phenylalanine. Scorbutic monkeys eliminated increased amounts of total tyrosine derivatives, keto acids, homogentisic acid, tyrosine and phenylalanine, and these excretions were further enhanced when they were fed tyrosine and phenylalanine.

Total tyrosine derivatives of liver, kidney, muscle, stomach, small intestine and adrenals of normal and scorbutic animals were estimated. Liver, kidney, muscle and small intestine of scorbutic monkeys contained more of these substances. Probably these tissues could not exidise tyrosine efficiently in ascorbic acid deficiency.

Nicotinic Acid-Tryptophan Metabolism in certain diseases.

Urinary excretion of nicotinic acid and amide, quinolinic acid, N'—methylnicotinamide, 6—pyridone, tryptophan, kynurenin, anthranilic acid and 3—hydroxyanthranilic acid was estimated in normal men and in patients suffering from typhoid fever, cholera, smallpox, cirrhosis of liver and infective hepatitis both before and 3 days after the feeding of tryptophan.

Excretion of tryptophan both before and after its feeding was diminished in all the diseases studied. Kynurenin which was absent initially in the urine of patients appeared in urine collected for 24 hours after the feeding in smallpox, liver cirrhosis and hepatitis but not in cholera. 3—hydroxyanthranilic acid appeared in urine after tryptophan was fed in cholera, smallpox and hepatitis but not in cirrhosis of liver. In all diseased conditions studied, except in typhoid and smallpox, urinary excretion of nicotinic acid and its metabolites was greatly diminished. After administration of tryptophan urinary excretion of nicotinic acid metabolites increased in all diseases but the increase was least in patients suffering from cholera. Patients suffering from smallpox excreted increased amounts of nicotinic acid and its derivatives both before and after tryptophan feeding as compared to normal men.

Since synthesis of nicotinic acid follows almost the rmal pattern in liver cirrhosis and hepatitis, it is unlikely that liver is the organ mainly concerned with this function. Diminished synthesis and metabolism of nicotinic acid in cholera might be due to deficient absorption of tryptophan from the intestine, or deficient synthesis of nicotinic acid by the intestinal bacteria which were washed away during purging, or deficient synthesis of nicotinic acid from tryptophan in tissues. In typhoid where the intestine is the chief site of lesion, the synthesis of nicotinic acid proceeds quite normally, which contradicts the belief that nicotinic acid is mainly formed in the gut. The enhanced rate of conversion of tryptophan into nicotinic acid and its final disposal in smallpox indicates that body tissues are more concerned in the synthesis of this vitamin

175. Enquiry on the metabolism of vitamins under Dr. J. Ganguly at the Indian Institute of Science, Bangalore.

A. *The intracellular distribution of esterase, vitamin A esterase, cholesterol esterase and of phosphatases in chicken liver :*

It is widely accepted that in rat liver homogenate vitamin A esterase and choleoterol esterase are quantitatively localized in the microsomal fraction alone. Homogenates of chicken livers were prepared in 0.25 M sucrose solution and were fractionated by means of differential centrifugation into the nuclear, mitochondrial, microsomal and supernatant fractions using similar procedures as used in the case of rat liver. Livers of male rats were treated under identical conditions and the fractions obtained from them were used as reference. In both species the acid phosphatase was concentrated in the mitochondrial, the glucose-6-phosphatase in the microsomal and the alkaline phosphatase in the supernatant fractions. In rat liver, esterase, cholesterol esterase and vitamin A esterase activities were to be found in the microsomal fraction only, while in chicken liver they were distributed between the nuclear and microsomal fractions. This variation in the intracellular distribution of the esterases might be due to species difference.

The pattern of distribution of free and esterified cholesterol and of proteins in the various cell fractions was almost identical in both chicken and rat liver, e.g., the esterified cholesterol was distributed between the microsomal and supernatant fractions, whereas free cholesterol was mostly in the microsomal fraction. The distribution of vitamin A ester and vitamin A alcohol was similar in both the species, the ester was entirely in the supernatant and the alcohol was mostly in the same fraction. In chicken liver, the carotenoids were distributed evenly among the mitochondrial, microsomal and supernatant fractions.

B. *Vitamin A esterase and cholesterol esterase :*

In the last year's report we compared the vitamin A esterase and cholesterol esterase activities of rat liver and concluded that they were not identical. Separation of the two activities can be achieved only if they are brought into solution ; but attempts at solubilizing them from the rat liver microsomes by using freezing and thawing, acetone, butanol, detergents, bile salts, etc. were unsuccessful. Both the nuclear and microsomal fractions of chicken liver are rich sources of these enzymes. On treatment of these cell fractions with Lissapol-N the nuclei yielded a clear solution containing the activities, but the microsomal enzymes remained insoluble. The Lissapol-treated solution was then subjected to electrophoresis on agar gels at pH 8.6 at low temperature for 24 to 48 hrs. In most cases the vitamin A esterase activity consistently moved faster than the cholesterol esterase activity, and in some cases the latter was totally lost leaving only the former activity. Thus, the three activities appear to be different.

C. *Absorption and storage of vitamin A and carotenoids :*

Animals display a great deal of variation in their ability to absorb and store different carotenoids; cows store mostly B-carotene, birds

mostly hydroxy carotenoids, man and frogs all carotenoids and most other mammals none. Chickens of both sexes and of unknown breeds kept on a low-carotenoid diet for two weeks, and normal adult rats were starved for 24 hours. They were then given oily solutions of vitamin A alcohol, its acetate or palmitate, with or without free-cholesterol, its acetate or palmitate, B-carotene, lycopene or lutein. The rats were killed 60-90 min. later, and the chickens 150-180 min. later by heart puncture. The small intestines were immediately removed and the contents washed out with physiological saline. The mucosal cells were scraped out with the blunt end of a scalpel from the intestinal muscles, and homogenized in 0.25 M sucrose and fractionated by differential centrifugation into th e nuclear, mitochondrial, microsomal and supernatant fractions. Irrespective of the form fed, vitamin A appeared mostly as ester in the intestinal muscle and in the supernatant fractions of the mucosal-cell homogenate, whereas cholesterol appeared mostly free both in the muscle and in the microsomal fraction of the mucosal cells. The concentration of the free cholesterol could hardly be increased in these fractions even after heavy dosing. In chicken lutein appeared in large concentrations in the intestinal muscles and in the mitochondrial and microsomal fractions of the mucosal-cell homogenate, whereas all the other carotenoids were present only in traces in the mucosal cells and in the intestinal muscles, in both species.

The carotenoids and vitamin A of chicken-liver homogenate were not extractable with diethyl ether by simple hand shaking with the solvent, whereas by treatment with various protein-denaturing agents and with trypsin and on freezing and thawing they became completely ether extractable. But there was a difference in their relative extractability following protein denaturation. The pattern of intra-cellular distribution of vitamin A and lutein is similar in both liver and mucosae. It is possible that separate stereo-specific lipo-proteins are present in dynamic equilibrium in the various tissues of chickens which specifically combine with vitamin A and lutein and thus help in their transport across the mucosal cells. This concept is in harmony with last year's report in which we suggested that separate stereo-specific lipo-proteins store vitamin A ester and vitamin A alcohol in rat liver.

176. Studies on the nutritive value of foodstuffs under Dr. M. V. Radhakrishna Rao at the Haffkine Institute, Bombay.

The work on the enquiry was commenced in June-August 1957 on the lines suggested by the Working Party of the Nutrition Advisory Committee appointed in this connection.

The following 19 foodstuffs have been analysed :

Cauliflower (*Brossica oleracea botrytis*) and Brinjal (*Solanum melongena*) from Baroda, Sangli and Bombay : Tur-tender (*Cajanus cajan*) and Papdi (*Phaseolus vulgaris*) from Baroda and Bombay; Maize tender (*Zea Mays*) ; Water-melon (*Cisrullus vnlgaris*) ; Jack fruit and seeds (*Ariocaipur heteropyllus*) ; Chauli small and big (*Vigna sinensis*) ; Peas-roasted (*Pisum sativum*) ; Bengal gram-roasted (*Cicer arietinum*) ; Ladies finger-big (*Abelmoschus esculentus*) from Bombay.

About 27 constituents were determined in these foodstuffs covering over 400 estimations. Tur, Papdi, Jack fruit and seeds, Chauli (both varieties), Feas and Grams are good sources of thiamine and riboflavin. Most of these foodstuffs are also high in sulphur content.

177. Studies on nutritive value of foodstuffs under Dr. B. C. Guha at the University College of Science and Technology, Calcutta.

The filling up of the gaps in Health Bulletin No. 23 is being continued. The following foodstuffs have been analysed for the nutrients, whose values are missing in the Health Bulletin and also for their copper, sodium, potassium, magnesium, sulphur, chloride, acid-base balance. oxalate, phytin phosphorus and available iron contents, which are not included in the Health Bulletin : Ladies finger (*Abelmoschus esculentus*), brinjal (*Solanum melongena*), pumpkin (*Cucurbita maxima*), calabash cucumber (*Lagenaria Siceraris*), spinach (*Spinaoia cleracea*), cauhfiower (*Brassica oleracea botrytis*), French beans (*Phaseolus vulgaris*), coriander leaves (*Coriandrum sativum*), bitter gourd, small variety (*Momordica charantia*), cabbage (*Brassica oleracea capitate*), carrot (*Dancus carota*), zhizyphus (*Zizyphus mauritiana*), peas (*Pisum sativum*), tomato, ripe (*Lycoperiscum esculentun*), beet root (*Beta vnlgaris*) lettuce (*Lactuca sativa*), neem leaves, tender (*Azadirachta indica*), mango, green (*Mangifera indica*), jambu fruit (*Syzigium cuminii*), rice raw, milled (*Oryza sativa*), rice, parboiled, milled (*Oraza sativa*), ridge gourd (*Luffa acutangula*), potato (*Solamum tuberosum*), bitter gourd (*Momordica charantia*), parwar (*Trichosanthes dioica, Roxb*), peas country (*Prunus, persica*), banana, ripe (*Musa pardisiaea*), *rohu* fish.

178. Studies on nutritive value of foodstuffs in the Northwest region of India under Dr. B. D. Kochhar at the State Food and Drug Laboratory, Ambala Cantt.

In setting programme for nutritional work in the Second Five Year Plan, the Working Party of the Council held the opinion that addition to the present knowledge of chemical composition of the foodstuffs used in different parts of India should form one of the objectives.

Studies on nutritive value of foodstuffs in Northwest region of India were therefore, undertaken at the State Food and Drug Laboratory (Ambala Cantt.);

The foodstuffs, were divided into broad categories like fruits, leafy vegetables, gourd-vegetables and grains (cereals and pulses).

With the necessary preliminaries completed beginning in March, 58' work wa started with fruits, in view of their availability in this season. So far 20s samples were analysed.

179 **Investigations on parboiling of rice under Dr. B. C. Gupta Dr. A. N. Bose at the University College of Science and Technology, Calcutta, and the College of Engineering and Technology, Jadavpur, Calcutta.**

The studies on the parboiling of rice on a pilot plant scale were undertaken after the fabrication and installation of the equipments designed for the purpose. The work so far carried out is described below :

1. *Soaking or Steeping of paddy* :

The alternative methods of soaking tried were as follows:

(a) Soaking in a vertical soaker with hot water a different temperatures circulated by a centrifugal pump,

(b) Soaking in water initially heated to 90°C 100°C, but without further heating the charge,

(c) Soaking with dilute solutions of different chemicals and

(d) Soaking with agitation in a continuous soaker-steamer.

For the first three processes, the time required for proper soaking 50 per cent moisture) was found to be almost the same. The rate of soaking in the agitating type of soaker (fourth process) was higher.

Lisapol (wetting agent) was tried but was not found to increase the rate of soaking although treatment with Lisapol after presteaming of paddy increases the rate of soaking.

2. The loss of vitaman B_1 due to leaching during soaking process was found to be negligible.

3. Soaking temperature was found to affect the colour of rice ; the higher the soaking temperature the more intense was the colour developed.

4. The effect of steaming time on the loss of vitamin B_1 of parboiled rice was found to be negligible but with the increase in steaming time the colour of the rice was more intense although the breakage due to subsequent milling decreased considerably.

5. Digestibility experiments *in vitro* on two commercial varieties of parboiled rice indicated that in one variety of rice it increased due to parboiling but in another case it decreased.

6. The changes in free nitrogen, amino acid nitrogen and reducing sugars are now being studied. Preliminary experiments indicate that with parboiling either the amino acid nitrogen or the reducing sugar content decreases depending on the variety of rice tested.

180. Studies on the Growth and Physical Development of Indian Infants and Children Under Prof. D. N. Majumdar at the Lucknow University, Lucknow.

In the Seminar held in September 1957 at Lucknow the Working Party revised the sampling procedure as well as the tabulation criteria. The material collected during 1957-58 was in conformity with the revised scheme. Lucknow Unit is a two team unit, one team consisting of lady anthropologist, a lady medical officer and a lady social worker for obtaining measurement on girls and the other of male members for obtaining similar data on boys. During the period under report, the male team completed examination of 4,539 boys. The ladies team, howevere, could not increase the number due to resignation of the authropologist nad medical officer. New members were recruited on October' 58 and it is hoped that the arrears in the examination of girls will be made up soon. The work on age verification could not be done systematically till the social workers were appointed. Now the ages are being verified for 25 pc. of the cases that are measured both in urban as well as rural areas. Care was taken to see that subjects from all the age groups were selected for age verification. In the rural areas, it was not possible to get any documentary evidence, i.e., horoscope or birth certificate, etc. To verify the age the elderly people of the village, however, correlated the birth of the child with other events in the family and this gave ages correct to months. No better assessment of age was possible. For urban areas documentary evidence was forthcoming in about 50 pc. of the cases selected for age verification. The tendency of keeping correct age records varied from district to district and from one religious group to another depending probably upon cultural traditions. Besides this, there was a tendency to round off the reported age in the neighbourhood of certain digits.

To get information regarding the economic status of the family in the rural areas, auxiliary methods were adopted. Home contacts became essential for this aspect of the survey as well as for other items like the environmental conditions, etc,. At times land records for the village were also examined for this purpose.

181. Cross-sectional studies of growth and physical development of normal healthy Indian infants and children under Dr. J.N. Berry at the Medical College, Nagpur.

The unit at Nagpur has to survey 8 districts of Vidarbha and 5 districts of Marathwada (in Bombay State) covering 20,000 subjects, 10,000 males and 10,000 females from 0 to 20 years age-groups. As per schedule, the unit has to cover 2000 children (of both the sexes) from Nagpur city.

The work commenced from 21st July 1958. First of all, a list of all the high, middle, and primary school in Nagpur, with their respective strengths on roll, was collected from the Office of the District Inspectors of Schools. The schools were divided arbitrarily into three socio-economic groups, i. e., High, Middle, and Lower. The number of children to be taken from each economic group was in proportion to the school-population in each economic group. Thus knowing the number of subjects, to be taken from each stratum, the technique of stratified sampling was followed and it was assumed that the age groups from 10 to 17 years would be available from the High Schools and 6 to 10 years from the Primary Schools. The required number of students was selected randomly from the selected schools giving due consideration to all classes. Before taking the measurements, the students who were medically unfit, were eleminated.

The subject of both the sexes from age groups 6 to 17 were taken from schools. For age groups 4 to 6 years Kindergartens were chosen and the required number was taken from them. For the remaining age groups i. e., below 4 years, house to house survey was done.

The data collected so far is being analysed. The unit has covered 1660 subjects up to the end of December 1958.

182. Studies on growth and physical development of Indian children Under Dr. P. Tirumala Rao at the Andhra Medical College, Visakhapatnam.

SAMPLE COVERED SO FAR

During the period Jan., 58 to Dec., 58, 898 boys, and 1,019 girls (Total 1,917) from birth to 20 years are examined from the city of Visakhapatnam whose population is 1,08,042.

Measurements were obtained on all items except subcutaneous tissue and blood pressure for which the supply of the necessary instruments is awaited. Classification of the measurements separately for both sexes is being carried out according to age and per capita income per annum.

In addition, a group of 463 unhealthy children from the low income groups was also examined. The aveage values of the various measurements in this group were found to be considerably lower than the values obtained in the regular survey, which included only those children who were apparently healthy.

183. Studies on growth and physical development of Indian infants and children under Dr. M. V. Phadke at the Sassoon Hospital, Poona.

The present investigation is a part of an All India Survey on Physical Growth of Indian Children under the auspices of I.C.M.R. The aim is to collect reliable scientific data on the normal growth patterns of healthy Indian children and also to study the effects of various socio-economic factors on these patterns of growth.

The area to be covered by the Poona Unit comprises the Marathi Speaking Districts of the old Bombay State covering a total population of 1,65,84,345 according to the census report of 1951.

Though the scheme was sanctioned in March, 1957 actual work commenced only in November, 1957 after the personnel were recruited and trained and the equipment made available.

The team has, so far, covered a part of Poona City and the surrounding rural area, Talegaon and part of Sholapur City. The work accomplished during the period October, 1957 to September, 1958 is as follows :

Station	Period	Number of children examined
Poona	5-11-1957 to 22-3-1958 29- 5-1958 to 31-5-1958	950
Chinchwad	2- 5-1958 to 10-5-1958	150
Talegaon	2- 6-1958 to 17-6-1958	200
Sholapur	30- 6-1958 to 30-7-1958	872
	Total	2,172

Information regarding age, living conditions, socio-economic status, etc., of all these was collected and properly recorded. The prescribed measurements were very meticularly carried out. All the data so accumulated has now been consolidated for further processing.

184. Studies on growth and physical development of Indian infants and children under Dr. (Miss) L. V. Pathak at the G. R. Medical College, Gwalior.

This unit is expected to cover the region of old Madhya Bharat and Bhopal, now included in the state of Madhya Pradesh. According to the instructions contained in the statistical note prepared by the Working Party, 12,500 children from the urban and 7,500 children from the rural areas have to be surveyed.

So far, an urban sample of 2,100 and a rural sample of 800 were examined from the city of Gwalior and its surrounding rural areas respectively. For the city sample about 15 different institutions were visited, while for the rural sample 8 villages were visited so far.

All the information contained in the Schedules is obtained with due care. The measurements were carried out strictly according to the instructions given. Since the work is in progress, no attempt has been made to analyse the data in detail.

185. Studies on growth and physical development of Indian infants and children under Dr. P. C. Biswas at the University of Delhi, Delhi.

With a view to obtaining norms of growth for Indian children from birth to 20 years of age, a cross-sectional study has been undertaken, covering the states of Delhi, Punjab, Rajasthan and Jammu and Kashmir as part of an all India Survey. The effects of various factors like economic status, place of origin, diet, etc., are also being studied.

About 40,000 healthy children are expected to be covered from this zone, chosen from a number of cities, towns and villages spread over the whole area. In urban areas, the required sample size in the age groups 5–20 years., will be obtained from schools and colleges, whereas samples for the age groups 0—5 years and the non-school-going section of the age groups 5—20 years will be obtained by means of house to house survey. On the other hand, the rural sample will be obtained by complete coverage of children in the selected villages, equal representation being given to the two sexes as far as possible.

During the period under report, a total number of 4,709 cases were studied—3,193 from Delhi, 966 from Simla and 550 from Chittorgarh (Rajasthan).

Four Anthropometric measurements were taken on each child *viz.* (i) stature, (ii) sitting height, (iii) weight and (iv) intercristal diameter. Information regarding the socio-economic background of the subject was also collected. Age in the case of school-going children was obtained mainly from school records, and in view of the importance of accuracy of these records, 10 per cent of the cases were verified by contacting the parents personally.

186. Enquiry into the somatotyping of male college students in Nagpur under Dr. J. N. Berry at the Medical College, Nagpur.

Somatotyping of one thousand male college students in Nagpur on the lines indicated by Sheldon *et al* (1954) and Tanner *et al* (1951) is being carried out. Besides establishing the basic physique patterns and the distribution of androgyny or femineity of build in the male this work should form the framework of studies for distribution of physique patterns in different racial and social groups and their relationship to diseases particularly cardio-vascular diseases. It would also help in finding out the changes if any, in the somatotypes brought about by age. By including all the male college students in a class it is expected that the sample would serve as a fair representation of male college students. in Nagpur.

The method of photographing the college students with a 35 mm. Cannon VT. Camera has been dealt with in detail in last year's report 580 male students from the Medical College, Nagpur, and 450 male students from the College of Science, Nagpur have been photographed. Total number of students photographed up-to-date is 1,030.

The work of photographing the requisite number of students is completed. Further analysis is held up for lack of photographic printing paper. It would be possible to determine the samototypes only when the prints are made from the negatives now obtained.

187. Investigations on lathyrism under Dr. (Mrs.) K. T. Ganapathy at Gandhi Memorial Hospital, Rewa.

The objects of the study were described in the previous reports. During the current year the following investigations were made.

(a) A complete clinical and biochemical study of 8 new cases in addition to the 16 covered earlier.

(b) A diet survey of 35 families of lathyrism patients and a nutrition survey of 419 subjects from villages where cases have been found.

(c) Collection of 38 sample 'birra' mixture, (a flour made of wheat, Bengal gram and lathyrus sativus in varying proportions from the houses of lathyrism cases, and

(d) post mortem on a doubtful case for subsequent study of the material by Dr. C. G. S. Iyer, Bombay.

Clinical Studies of Lathyrism.

Age incidence.—Analysis includes all the 24 cases observed so far. Maximum number of cases occurred between 21 and 40 years.

Sex incidence.—Out of 24 cases, only 3 were females and 21 males.

Family incidence.— 8 out of 24 gave history of family incidence.

Seasonal incidence.—The highest incidence of the disease was observed in the months from July to September.

Mode of onset.— Out of 24 cases 18 gave history of acute onset of the disease and 6 were said to have developed the disease gradually.

Precipitating factors —The precipitating factors in 12 cases were heavy exertion and exposure to moisture. In 5 cases it was precipitated by febrile illness while in 7, no such factors were observed.

Physical examination.—The general appearance of the patients was good. The postures and gait usually depended upon the duration and stage of the disease. An early case might sometimes go with a normal posture Pain in the lumber region was complained before the onset of the disease. Involvement of cranial nerves and sensory disturbances were not found in any case. Lower abdominal and cremastric reflexes were lost in most of the cases. Muscular rigidity in the lower extremities was a common finding. Adductors of the thighs and dorsiflexors of the ankles was generally weak. Knee and ankle jerks were exaggerated. Patients walked with a crossed gait and knee joints flexed. In some cases where the tendo-achillis gave way patients could not walk on toes, but the bending at knee joints became more prominent. Rombergism was always negative.

Haematology.—30.8 per cent of the total cases showed microcytic hypocromic type of anæmia. Out of 24 cases, 16 showed normal curves for total and free gastric acidity. Five cases of alchlorhydria and 3 hypochlorhydria have been recorded. Sternal puncture examination was normal.

Dietetic back ground.—All cases gave history of consumption of lathyrus sativus since their childhood. Chappaties were prepared out of the mixture (birra) consisting of varying amounts of lathyrus sativus. Vegetables, fruits, sugar, jaggery, ghee, milk and its product were only occasionally consumed in small quantities.

Biochemistry.—Blood biochemistry did not show any abnormal changes except that serum calcium in all the cases was high ranging from 10.50 mg. to 14.00 mg. per cent with an average of 12.32 mg. per cent C. S. F. was normal in all respects except for the high protein which ranged from 41.35 mg. to 78.01 mg. per cent with an average of 53.01 mg. per cent.

Early cases.—Three early cases were investigated. Early cases were only those who showed symptom-complexed with or without minimal signs of lathyrism. Two cases recovered completely when they were taken off their home diet and kept on hospital diet. The third case did not show any improvement. She was then kept on vitamin A Tab. (50,000 I. U.) for two months with no significant improvement.

FIELD STUDY OF LATHYRISM.

(i) *Diet survey.*—Diet survey was carried out by weighment of raw foods on 35 families of lathyrism patients. Each family was observed for a period of 7 days. The surveys were carried from November, 1957 to July, 1958. The consumption of pulses other than lathyrus sativus in the families of lathyrism patients was high, ranging from 9.9 oz. to 17.8 oz. per consumption unit per day. The consumption of lathyrus sativus ranged from 3.8 oz. to 16.5 oz. per consumption unit per day.

The results showed a high consumption of vegetable proteins, ranging from 209.1 g. to 267.3 g. per consumption unit per day. The figures for calorific value were also high (3410 to 5430).

The average monthly income per family varied from Rs. 31/- to Rs. 98/-. The number of consumption units per family varied from 2.5 to 7.5.

(ii) *Nutrition survey.*—Four hundred nineteen subjects were examined in the families where diets were surveyed. Deficiency diseases caused by avitaminosis A & C were the commonest features.

(iii) *Ameliorative measures.*—Distribution of multi-vitamin tablets in the populations surveyed, was undertaken. A film on lathyrism was shown to educate the people on the bad effects of *Khesari dal*.

(iv) *Collection of samples of lathyrus sativus.*—Thirty eight samples of mixtures (birra) consisting of wheat, Bengal gram and *Khesari dol* were collected from the households of lathyrism patients and sent to Dr. Sadashivan, University of Madras and Nutrition Research Laboratories, Coonoor, for analysis. The quantity of lathyrus sativus in the samples of mixture (birra) ranged from 4 per cent to 95.2 per cent with an average of 38.2 per cent.

Note:—New observations on hypercalcæmia and high C. S. F. protien (normal chlorides, sugar and cell count) have been recorded. High consumption of pulses and vegetable protiens have also been found in the families of lathyrism patients.

188. Investigations on lathyrism under Prof. T.S. Sadasivan at the University Botany Laboratory, Madras.

Semi-quantitative spectrochemical analyses of *L. sativus* seeds and other common edible legumes were made for Ca, Mg, K, Mn and Sr. Quanta of Ca were much higher in *L. sativus* seeds than in the other legumes tested. This factor probably explains the hypercalcemia in the lathyrism patients. Further work along this line is indicated. Manganese in other legumes was very low, while in a few of the *Lathyrus* seeds samples it was appreciably high, the highest value recorded being 14.0 mg. per 100 g. of dry seeds. As Mn toxicity is known to cause nervous disorders resembling Parkinsonism, further quantitative determinations of Mn in a large number of seed samples by a highly sensitive spectrophotometric method, using ammonium persulphate oxidation process, were made. Three out of 33 samples tested showed 42.0, 49.5 and 50.0 mg. of Mn in 100g. of dry seeds, four more samples showed 10.9 to 23.5 mg. and in the rest it varied from 1.8 to 7.5 mg. On the contrary in the case of the other commonly used pulses, the amount of Mn was below 3.0 mg. In view of the possible cumulative Mn toxicity, these data suggest the possibility of Mn being an aetiological factor in the causation lathyrism. Experiments will now be undertaken to screen more samples of seeds and soils from the field and to elucidate in what form(s) (whether manganous or manganic) Mn occurs in the seeds.

A series of greenhouse experiments were made to grow *L. sativus* plants, under controlled conditions in garden soil and *L. sativus* field soil obtained from Bihar. While there was good percentage of germination in both soils, plants failed to grow beyond the seedling stage in the garden soil while in *L. sativus* field soil the plant growth was normal. This is indicative of the presence of favourable strain(s) of the symbiotic *Rhizobium* spp. in Bihar soils, necessary for the normal development of root nodules and plant growth. Further work on isolation, identification and reinoculation of soils with the *Rhizobium* isolates and their influence on the growth of *Lathyrus* plants is being undertaken.

Systematic screening of samples of *Lathyrus sativus* obtained periodically from the Field Unit at Rewa, for isolation of fungal flora and their identification is being continued.

189. Research on defluorinating substances applicable to water : development of a defluorinating process for practical application in areas of endemic fluorosis under, Dr. S. C. Pillai at the Indian Institute of Science, Bangalore.

In continuation of the earlier studies on the efficiency of fluoride removal from water by tricalcium phosphate, further work was carried out using other phosphates and phosphorus-containing materials. The phosphates employed for the experiments were aluminium phosphate, superphosphate, calcium phosphate prepared from superphosphate by treatment with lime (1:10 aqueous extract of superphosphate treated with lime water and washed free from alkalinity), alkali-treated superphosphate (treated repeatedly with 4 per cent sodium hydroxide until there was no dissolution of phosphate), bone and bone charcoal. The efficiency of a fewother materials was also studied. These included calcium aluminate, aluminium silicate, coral reef and a cation exchange resin prepared from the tannin-containing bark of *Acacia arabica* by condensation with formaldehyde.

The results of these studies showed that although aluminium phosphate, bone, bone charcoal, calcium aluminate, aluminium silicate and the tannin-formaldehyde resin removed fluorine from water to a certain extent (varying from 0.4 to 1.8 mg. of fluorine per g. of the substance), the materials prepared from superphosphate were more efficient (removing 2.0 to 2.4 mg of fluorine per g. of the substance). The latter appeared to be cheaper and more suitable for application in rural areas.

Preliminary experiments indicated the possibility of preparing a suitable defluorinating substance from phosphatic nodules (obtained from Trichinopoly, the material containing about 28 per cent P_2O_5) by treatment with sulphuric acid (1 gm. of the acid per g. of P_2O_5 in the nodules followed by treatment with 5 per cent sodium hydroxide.

190. Research on Endemic Goitre under Dr. V. Ramalingaswami at the All-India Institute of Medical Sciences, New Delhi.

The work done up to the present time was concerned mostly with the selection and standardization of methods for the estimation of Protein-bound iodine in the serum and with the estimation of uptake of ^{131}I in normal euthyroid persons.

A number of methods were examined and tested for estimating the protein-bound iodine in serum. Out of these, Barker's method, with minor modifications and using the wet micro-incineration procedure, has proved to be the most satisfactory.

The serum protein-bound iodine in twelve normal euthyroid persons was found to range from 3.9—5.8 μg. of iodine/100 ml. of serum with a mean value of 5.5. μg.

The ^{131}I uptake of normal euthyroid persons was investigated. 50 micro-curies of ^{131}I in a capsule was administered orally on an empty stomach to each person, along with two tumblers of water. Immediately before administering radio-iodine, a background count was taken of the thyroid region with a scintillation probe kept at a distance of 12 inches from the thyroid region of the neck. After administration of radio iodine, counts were taken at 2, 6, 24 and 48 hours after the dose. In five normal euthyroid persons, the uptake ranged from 15 to 25 per cent at the end of 24 hours with a mean value of 23.1. The first 24 hours sample of urine contained up to 70 per cent of admininstered radio iodine.

Methods for the estimation of iodine in the urine are being evaluated at the present time.

191. Indigenous drug enquiry at the School of Tropical Medicine, Calcutta.

I. Continuation of studies on the pharmacological action of alkaloids of Rauwolfia serpentina (R.S.) and some of the vehicles on the normal blood sugar level of anaesthetised cats.

The earlier preliminary observation that there is a difference in the effects brought about by the total alkaloids of R. S. and reserpine on the blood sugar of anaesthetised cats, has been confirmed.

One hundred ml. of ethanol extract of total alkaloids of R. S. (1 per cent) was dissolved in 2 per cent HCl and extracted into two parts (a) the resinous matter and (b) the resin-free total alkaloids. Each fraction was dissolved in 100 ml. of ethanol, and tested for their action on blood sugar level of cats under chloralose anaesthesia. Results were as follows :—

A. *Total resinous fraction* after 0.1 ml./kg. i. v., caused a mild increase of blood sugar level in anaesthetised cats.

B. *Resin-free total alkaloids* of R. S. caused a lowering of the blood sugar level after 0.1 ml./kg. i. v.

C. *Reconstituted total alkaloids*: Equal proportions of the above two fractions were combined together and the volume evaporated in room temperature, to half; this *reconstituted total extract* (1 per cent) also caused a lowering of blood sugar level after the same dose, viz., 0.1 ml./kg. i. v. This finding, with the reconstituted total alkaloids tallied with results reported last year with the original total ethanol extract.

D. *Serpine*: Serpine 0.5 mg./kg. i. v., caused an earlier and more marked fall of blood sugar level in normal anaesthetised cats than total alcoholic extract of R. S.

E. *Effect of some of the vehicles used for making solution of R. S. alkaloids :* Various vehicles have been used to make solutions of R. S. alkaloids and therefore a few vehicles have been tested for their action on blood sugar levels, to serve as controls.

(i) *Ethanol.*—Most of the extract of R. S. used in this laboratory were made with ethanol and hence the pharmacological action of ethanol on normal blood sugar of anaesthetised cats was studied. A dose of O. 1 ml./kg. i. v., ethanol was found to cause a mild lowering of the blood suger level.

ii) *Ascorbic acid.*—Preliminary results with ascorbic acid (Redoxon, Rocke) 15 mg./kg. were variable so far as its pharmacological action on normal blood sugar level was concerned.

(iii) *"Ciba" vehicle for Reserpine* (*Serpasil*, Ciba Ltd., Switzerland). This was obtained in original through the courtesy of Ciba Ltd., (Switzerland). . This agent used as a control, showed a mild lowering of the blood sugar level after 0. 1 ml./kg. i. v.

Thus both ethanol and the 'Ciba' vehicle were found to cause a depression in the blood sugar level.

II. *Continuation of work on diuresis of water-loaded rats.*

Effect of some indigenous plants. This investigation was carried out in two parts :—(1) exploration into the action on diuresis, of such indigenous plants which were claimed as anti-diabetics in indigenous medicine but the claims were not substantiated by modern methods, (2) screening of other indigenous drugs supposed to be diuretic, and comparing their effects, where necessary, with those of urea.

A. *Gymnema sylvestre* Br. (*Hind :* Gurmar, *Beng:* Gadalsinghi). Results of a preliminary investigation with the aqueous extract of this plant reported last year showed an anti-diuretic effect. Administration of a crude ethanol extract orally, was also found to cause a marked anti-diuretic effect which varied directly with the dosage used ; a higher dose of the extract sometimes caused a complete suppression of urine for five hours and the effect persisted, although to a less degree, for as long as 24 hours.

B. *Dlichos biflorus*, Linn. (*Hind*: Kulthi).—Diuretic effect in rats was seen after oral administration of infusion from 1-4 g. of seeds/ kg. rat.

C. *Clitoria ternatea*, Linn. (*Hind :* & *Beng.* Aparajita). Infusion of whole plant produced slight diuretic effect.

D. *Hygrophila spinosa*, T.Anders (*Hind*: Kantakulia, *Beng*: Kulia-khara). Infusion prepared from immature whole plant produced no diuretic effect.

E. *Achyranthus aspera* Linn. (*Beng:* Apang, *Hind*: Datjira). A decoction of this plant produced diuretic effect.

F. *Boerhaavia diffusa*, Linn. (*Beng* : Punarnaba). A preliminary experiment with infusion of whole plant orally produced diuresis in rats.

The diuretic potency of the above five drugs were compared with that of urea and the results suggested tentatively the following order of efficacy: *A. aspera*, *Cl. ternatea*, *D. biflorus*, *B. biffusa*.

III. *Pharmacological studies on Arborine.*

This alkaloid was isolated from the leaves of Glycosmls pentaphylla Correa (*Beng* : Ash-shoura, *Hind* : Banabimbu) by the Department of Chemistry, School of Tropical Medicine. This plant has been mentioned as an anti-dote to snake bite. Chemically it has a quinazolone grouping. Plants containing this 'grouping' are rare in nature and hence the importance of pharmacological studies.

The substance was fairly non-toxic to mice, but caused a brief fall of blood pressure in chloralosed cats, which was not affected by vagotomy or atropine and/or mepyramine. This effect was absent in spinal

cats. The muscarine-like action of acetylcholine on B. P. was slightly potentiated after a dose of arborine in chloralosed cats. Intestinal movements were also stimulated.

There was an increase in the rate, the amplitude of beat and the coronary flow of isolated guinea pig hearts ; the last action was quite pronounced and none of these components were affected by mepyramine, whereas, of the similar effects brought about by histamine, the increase in coronary flow was antagonised by mepyramine.

On isolated guinea pigileum there was no action, but a partial antagonism to the spasmogenic action of histamine and acetylcholine was seen after high doses of arborine. It caused occasional increased peristalsis and mild tonic contraction of isolated rabbit duodenum and the higher doses slightly potentiated the relaxation caused by adrenaline.

On isolated guinea pig seminal vesicle arborine in high doses slightly antagonised the spasmogenic action of adrenaline and histamine. Spasmogenic action of acetylcholine and pituitrin on the isolated rat uterus was completely antagonised by it. A neuromuscular blocking activity was exhibited in a preliminary experiment on the cat's gastrocnemius-sciatic nerve prepration after intra-arterial injection of arborine.

In water-loaded rats, intraperitoneal arborine in low doses (5-10 mg./kg.) increased the percentage of water excretion while high doses (80 mg./kg.) diminished it and produced methaemoglobinurea.

192. Study on the anti-veratrinic, anti-accelerator and anti-arrhythmic activity of indigeneous and synthetic drugs and on the action of anaesthetics and pre-anaesthetics on cardiac automaticity and conductivity under Dr. R. B. Arora at the All-India Institute of Medical Science, New Delhi.

1. In 22 dog-experiments on *Cardiac Automaticity*, the blood concentration of ether which produced disappearance of P wave in the EKG was determined. The P wave disappeared temporarily in 40 per cent of cases at a blood concentration. Varying from 90-172 mg. per cent. Since the usual concentration of ether in the blood of human beings in the 3rd stage of surgical % anesthesia is 120-150 mg. disappearance of P wave may occur in some cases and this may lead to the development of cardiac arrhythmias. A constant watch, therefore, may have to be maintained on the rhythm of the heart during ether anesthesia in human beings.

2. Jatamansone and some antimalarial drugs of proved anti-arrhythmic value were tested for their suppressor effect on the ectopic impulse, in 48 unanaesthetized coronary dogs with ventricular tachycardia. This arrhythmia etiologically resembles a type of arrhythmia occurring in human being following myocardial infarction, and permits the observation of the side effects in a conscious animal which is a distinct advantage. Jatamansone chloroquine, plaquenil, primaquin and isopentaquine were more effective, while paludrine was less effective than quinidine. Camoquin, however, was totally devoid of any action. Except isopentaquin, no other drugs produced any demonstrable toxic effects in the unanaesthetized coronary dog.

3. To further test the concept of unity of fundamental mechanism of excitation in nervous and cardiac tissues, four more drugs with anticonvulsant action (chlorpromazine, primidone acetazoleamide, phenylglycodol), were tested for ectopic-impulse-suppressor-effect in 30 unanaesthetized coronary dogs. Chlorpromazine and primidone were more effective than quinidine in this respect. No toxic side effects were noticeable in the anaesthetized coronary dog. As a corollary to the above, some drugs which had been tested in the coronary dog (crescinnamine, serpentine, ajmaline and jatamansone) were also tested for their anticonvulsant activity against maximal electro shock and metrazol seizures in 328 albino rats. It was found that drugs which did not possess an antiepileptic action, were also devoid of activity in coronary dog and *vice versa*. A further study of this phenomenon using some more drugs, may throw light on brain dysrhythmias and its similarity, if any with dysrhythmias of heart.

4. In 10 experiments on dogs, the production of atrial arrhythmias resulted in increased serum potassium levels. This indicated an efflux of potassium from the myocardial cells. Quinidine-induced reversion of traumatic atrial flutter, and spontaneous reverion of acetylcholine induced atrial fibrillation, resulted in the decrease of serum potassium to prearrhythmic levels. This provides a direct evidence for potassium efflux in atrial arrhythmias, as against the indirect evidence adduced by Burn *et al.* (1955), Leveque, (1957) and Holland, (1957.)

More experiments are being carried out at present on this aspect. The role of potassium flux in ventricular arrhythmias and its modification by anti-arrhythmic drugs will now be studied.

5. A total of 19 heart-lung preparations of dogs were used to study the anti-accelerator action of five antimalarials: Anti-accelerator activity of plaquenil and premaquin was found to be superior to that of quinidine. Their I-50 values were found to be 50 mg. in each case. Camoquin, mepacrine and isopentaquin were found to be devoid of anti-accelerator action. Since dynamics of circulation are affected by cardiac arrhythmias associated with a rapid heart rate, the anti-accelerator action of anti-arrhythmic drugs may be of clinical significance. Other drugs which were found superior to quinidine in experimental cardiac arrhythmias are being investigated.

6. The technique of inducing arrhythmias with camoquin and epinephrine has been fully established. Camoquin in a dose of 5 mg./kg. i.v., followed by epinephrine 2 ug./kg. i.v. resulted in ventricular arrhythmias in all the cases. Both quinidine and epinephrine when injected separately in these dosages did not produce arrhythmias. Quinidine in a dose of 5 mg./kg. effectively prevented the development of this arrhythmia in 20 dogs.

7. As a result of earlier studies on 45 compounds and recent studies on 16 compounds the anti-veratranic activity, as a method of screening anti-arrhythmic drugs has been fairly well established. An action on refractory period and/or conduction does not appear to be essential for antifibriallatory activity since emetine and procaine do not effect the former but still possess an anti-veratrinic action in concentrations of 1 : 100,000 and 1 : 30,000, respectively. All drugs effective in atrial arrhythmias also possess an anti-veratrinic action. No such correlation between anti-veratrinic and anti-arrhythmic actions is found in the case of drugs effective in ventricular arrhythmias.

193. Enquiry into the influence of anti-reheumatic substances on the hypothyse o-adrenocortical axis under Dr- G.K. Karandikar at the Medical College, Baroda.

Sodium salicylate 0.3 g./kg. i.p, depleted only the ascorbic-acid content of the adrenal gland at the end of 2 hours and 6 hours. Butazolidine 50 mg./kg. depleted only the cholesterol content of the adrenal glands in 2 hours and produced only eosinopenia at 4 and 6 hour intervals. Colchicine 1 mg./kg. depleted only the cholesterol content of the adrenal glands at the 4th and 6th hours. Aminopyrine 150 mg./kg. depleted the cholesterol content of the adrenal glands and produced eosinopenia at the 2nd hour. Mahayograj Guggal, 100 mg./kg. orally, produced eosinopenia at the 2nd and 4th hours. One Maharasana decoction 20 mg./kg. orally, produced eosluspenia at the 2nd and 4th hours.

Sodium salycylate, 0.3g./kg. orally, Mahayograj Guggal, 100 mg./kg. orally, and Maharasana Adi Quath, 20 c.c./kg. orally, produced an anti-inflammatory action.

194. Enquiry on the anti-convulsant and antifibrillatory action of drugs in relation to acetylcholine synthesis under Dr. B C. Bose, at the M.G.M. Medical College, Indore.

The work on the above enquiry was undertaken with a view to study the mechanism of action of anti-convulsant and antifibrillatory groups of drugs with particular reference to acetylcholine content in brain tissue. Inhibitory effect of anti-convulsant drugs on acetylcholine synthesis was reported last year. The effect of antifibrillatory drugs, chlorpromazine, promethazine and quinidine-on acetylcholine content in rat brain has been investigated. Frog rectus method following the desensitization technique of Feldburg has been used for the estimation of acetylcholine in brain extracts. It has been observed that chlorpromazine inhibited acetylcholine synthesis in brain tissue of rats to the extent of 24.5 to 29 per cent in a dose range of 1-2 mg./kg. Promethazine has been found to be more potent in this respect, producing 46.5 per cent reduction in acetylcholine content in a dose of 1.5. mg. 6 mg./kg. The observation quinidine indicated that this also inhibited the biosynthesis of acetylcholine in brain, although to a very small extent compared to that of chlorpromazine and promethazine.

A series of investigation has also been undertaken to study the protective effect of these drugs on the minimal shock convulsive threshold in rates. Chlorpromazine in a dose range of 2·5 to 5 mg./kg.has been found to lower the threshold initially but raised it subsequently, while promethazine (0.35 to 23 mg./kg.) produced significant protection, the action persisting up to 120 minutes. Quinidine and paludrine have raised the M. E. S. T. to a slight extent only.

An increase in 5HT level in brain after administration of anti-convulsant drugs was reported by Bombay Castle (1957).the investigation on minimal shock threshold in rate was extended to study the roil of 5HT singly or in combination with the above drugs. It was observed that 5HT in a dose of 50 μg./100 g. raised the normal M.E.S.T., when this was used along with chlorpromazine promethazine or an anti-convulsant drugs, an initial rise in the threshold with subsequent lowering was observed. In the case or milontin a consistent increase in the threshold was noticed. The observations indicate that 5HT exercise a synergestic action with anti-convulsant and antifibrillatory drugs toa certain extent.

195. Enquiry on serum electrolytes (Na and K) under different experimental and climatic conditions in normal human adults permanently resident in the Punjab plains under Dr. Shiv Kumar at the Medical College, Amritsar.

Serum sodium and potassium levels were estimated with the help of Eet Flame Photometer in 70 apparently normal healthy adults aged between 18 and 22 years. Post-absorptive specimens of blood were collected in all the cases for analysis.

In the present study the range of serum sodium, as determined by the Flame Photometer method, varied between 130.0 and 158.0 mEq/L with a mean of 141.1 mEq/L. The serum potassium levals ranged between 3.6 and 5.7 mEq/L. with a mean of 4.4 mEq/L. The serumr sodium levels as determined chemically and by Flame Photometric methods, showed no significant difference. In the case of potassium the difference in the results by the two techniques appear to be significant. The Flame Photometer technique was probably more precise. The chemical method gave wider scatter and false high values in certain cases.

The values for Indians obtained in the study compare well with those reported from the West.

In the present study the estimations were done during the summer months and the work is in progress to see if there is any change in the levels during the winter..

Diet, weight, and height seemed to have no effect on serum sodium and potassium levels.

196. Study of anthelmintic action of shell oil of cashewnut under Dr. N. V. Bhaduri, at the School of Tropical Medicine, Calcutta.

The anthelmintic action of the coshewnut shell oil against the hookworms roundworms and whipworms of man was first observed on small number of cases, by Eichbaum and his associates in 1950 in Brazil. Bhaduri and Sanyal made preliminary observation with an extract prepared by one of them (P.K.S.) in 1952 and found encouraging results against *Arcaris*, hookworms and *Fasciolopsis buski*. No action was observed against *Trichuris*.The drug was well tolerated and non-toxic. Further work by Bhaduri and his associates in 1953, 1954 and 1956 showed that the drug was also effective against, *Hymenolepis nana H. diminuta* and possessed some degree of anthelmintic action against *Enterobius vermicularis* and *Strongyloides stercoralis*. Against *Trichuris, Taenia solium* and *T. sanginata* no action was observed. The draw back of the drug was that it was an irritant liquid and had to be put in hard gelatin capsules. To overcome this difficulty the drug was mixed with milk and given to patients. The mixture was non irritant but much of the anthelmintic action was lost.

The study of the chemical make-up of the extract was next taken up. The extract contained, cardol, cardanol and a small amount of anacardic and some neutral substances. As cardol was similar to hexylresorcinol, tois was first taken up for study of its anthelmintic action. Cardol, similar to the whole extract in appearance and irritant properties, was found to be a good anthelmintic, and its range of action was like the whole extract, i.e., it was active against hookworms, *Ascaris, Hymenolepis nana, Enterobius vermicularis*. It showed some action against *Trichutis tristrichiura*. The drug was well tolerated and non-toxic and could be used on patients suffering from various ailments even when their general condition was not very much improved.

Cardanol too showed anthelmintic action against hookworms, *Ascaris* and *Hymenolepis nana* and *Enterobius vermicularis* but was weaker.

Unfortunately cardol and cardanol were both irritant like the whole extract and could not, therefore, be used in children on a large scale.

Attempts were, therefore, made to prepare solid derivatives from the extract or its fraction to obtain a similar good anthelmintic.

By a specially devised method hydrogenation, tetrahydrocardol for tetrahydro anacardic acid were prepared in the laboratory for further study.

Tetrahydrocardol was a powder, insoluble in water and exhibited some degree of anthelmintic action but was weaker than the whole shell extract or cardol. It was, however, well tolerated and non-toxic.

The study of the anthelmintic action of tetrahydro-anacardic acid was begun in 1958. This compound was also found to have some degree of action against the hookworms and *Ascaris* but was much weaker than the whole shell extract or cardol.

Various compounds were also prepared from cardol and anacard acid; these were, sodium anacardate, calcium anacardate, magnesium an cardate, aluminium anacardate, sodium salts of cardol and shell extra and acetylated derivatives of the hydrogenated products of cardol, card anol and anacardic acid.

The study of the anthelmintic action of the prepared drugs is progress and observations have been made on only a small number of cas with calcium anacardate, magnesium anacardate, aluminium anacarda and these have been found to have some degree of anthelmintic acti against hookworms and *Ascaris*.

Together with this the effect of using sodium anacardate with card (which is similar to hexylresorcinol) is also being studied.

In a dose of 3 g., sodium anacardate did not appear to be of mu value against hookworms or *Ascaris*. Used together with 6 g. of card it, however, cured 10 out of 12 cases of ascariasis and the combin therapy was well tolerated. Out of 5 cases of *Hymenolepis nana* 3 we cured. Out of 27 cases of hookworm infection treated, 6 cases we absolutely cured and 8 cases, practically cured i.e., 14 cases were cured nearly completely cured. In the whole group the reduction in the eg count (which may be taken as worm expulsion rate) was 80 per cent— encouraging result.

197. Enquiry on canine and human gastric function in summer and winter under Dr. B.S. Kahali at the S.M.S. Medical College, Jaipur.

Human gastric function :

Investigation on thirty randomly selected healthy volunteers, aged between 18 and 28 years have been completed during winter, summer and rainy seasons.

The method used was that of fractional gastric analysis fo Rehfus as modified by Napier and Dasgupta. Three test meals were tried-oatmeal gruel found pectin meal and 7 perceen tethyl alcohol. Ethyl alcohol was found to be the most suitable and was used in all the experiments.

Free acid, total acid, total chloride and pepsin were estimated in resting juice as well as in samples collected after the test meal.

Pepsin was estimated by a modification of the technique of Hunt (1948) with human plasma or serum protein as substrate. Other protein substrates, commercial egg white, fresh egg white, casein, and bovine serum albumin, have been used, but were found unsatisfactory. For free and total acids and total chloride the methods recommended by Napier and Dasgupta were used. Blood chloride was also determined by the standard method. There was a considerable day to day variation in gastric secretory response and hence a single analysis of the gastric secretion would be of limited value for estimating the secretory activity of the stomach. In this investigation the average of three determinations was used to estimate the functional efficiency of the stomach.

The results of the investigation during the three seasons were analysed statistically.

The acid secretory response of the stomach to the alcohol test meal was significantly more in winter and rainy seasons than in summer.

There was no statistically significant variation in the pepsin content during the three seasons.

There was no seasonal variation of the basel secretion. Basal Secretion also showed considerable day to day variation. There was no correlation between the basal and the post-list-meal secretory activity. Pepsin and acid varied indeperdently of each other.

The motor functions of the human stomach were studied with the aid of *x-rays*. The findings indicated that evacuation time was depressed moderately in summer compared to the motor functions in winter.

Canine gastric function :

The investigation was carried out on dogs after making an upper gastrostomy opening. This method was very useful as the operated dogs remained healthy and normal for prolonged periods of observation.

Animals with innervated gastric pouch did not survive long.

The gastric secretion induced by injecting histamine (0.06 mg./kg.) parenterally was collected for half an hour through the gastrostomy opening by aspiration with a catheter. The contents were analysed by the standard methods. Blood sodium, potassium and chloride were also estimated. Seasonal variation is being studied in these animals.

The effects of hormone administration and certain physical conditions on histamine induced gastric secretion, were studied.

(i) Acute exposure to both moderately cold and warm environments had a depressant effect on the volume and acidity of secretion.

(ii) Thyroid medication depressed the secretion. Volume of secretion was diminished. This may be due to greater motor activity of the stomach induced by thyroid.

Desoxycorticosteryne increased gastric acidity ; volume however, was diminished.

(iii) Adrenocorticotropic hormone also induced hypersecretion of acid but the effect was less than that induced by desoxycorticosterone. The volume of the secretion was, however, diminished.

(iv) Combined thyroid and adrenocortropin medication induced increased secretion of acid associated with decrease of volume,

Thyroid did not cause any change in the blood sodium, potassium and chloride levels.

Desoxycorticosterone increased blood sodium and chloride and lowered the potassium level.

ACTH caused a rise of sodium and chloride but to a lesser extent, potassium level, however, remained at the normal value.

Combined thyroid and adrenocorticotropin induced slight hypernatraemia and hyperchloraemia and hypo-kalaemia.

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198. Neuro-physiology research unit under Dr. B. K. Anand at the All-India Institute of Medical Sciences, New Delhi.

The work done in this unit is being presented in two sections, one dealing with the neuro-physiological studies, and the other dealing with the physiological studies on yogis.

I. Studies on Neuro-physiology

(1) *Nervous regulation of food intake :*

It has been reported previously that the "*hunger*" mechanisms (both "feeding" as well as "satiety") are located within the hypothalamus while the discriminative "*appetite*" is in the forebrain. Studies have been carried out to test the mechanism of these centres.

(i) *Action of blood sugar levels on hypothalamic "feeding" centres:*— In 15 cats and 17 monkeys electrical activity of different hypothalamic (including "feeding" and "satiety" centres) and neocortical regions was recorded through implanted depth electrodes on an 8 channal Grass Electro-encephalograph. Hyperglycaemia or hypoglycaemia was then produced by either glucose saline or insulin given intravenously and their effects on the activity of these centres noted. It was observed that hyperglycaemia probuced decrease in the activity of the "feeding" centre and increase in the activity of the "satiety" centre on the other hand, hypoglycaemia increased the activity of 'satiety' centre. No change in activity could be observed in other hypothalamic and neocortical regions. It is thus observed that changes produced in blood sugar (and probably in certain other components of blood) as a result of feeding, may be instrumental in regulating the activity of these 'feeding' and 'satiety' centres.

(ii) This work is being extended to study the effect of changes in blood amino-acid content and fat content on the hypothalamic centres.

(iii) The effects of such drugs as Reserpine and Restinon (which increase appetite) on the activity of hypothalamic centres is also under investigation.

(iv) Evoked-potential studies are also being carried out to find out whether the sensations originating in the stomach project to hypothalamic centres and thus play a part in regulating the activity of these centres.

(v) The activity of those limbic regions, which previously have been shown to infiuence food intake, is also being recorded electro-encephalographically under the influence of changes in blood chemistry.

(2) *Nervous regulation of water intake:*

Hypothalamic regulation of water intake has already been reported. Influence on water intake of those regions of the limbic system which effect the food intake, is being studied by producting discrete lesions in them in albino rats.

(3) *Control of Visceral and Metabolic activities from the Limbic System of the Brain :*

In 21 monkeys simultaneous recordings of different visceral responses were taken on an Offner Type T 8 channel electroencephalograph during stimulation of different limbic structures.

(i) *Visceral responses :*

(a) *Blood pressure* .—Stimulation of most of the regions produced a fall ; but there was some rise when stimulation was restricted to the orbital surface of the frontal lobes, the temporal tips and the amygdaloid regions.

(b) *Heart rate* .—Increase was observed after stimulation of amygdaloid, peri-amygdaloid regions and temporal tips. Stimulation of other regions produced variable results.

(c) *Hand volume changes* .—Temporal tip and hippocampal stimulation caused vasodilatation, whereas frontal lobe stimulation produced vaso-constriction.

(d) *Respiratory changes* .—Stimultation of orbital surface, temporal tip, hippocampal gyrus and amygdaloid peri-amygdaloid regions produced slowing and arrest.

(e) *Skin temperature* .—Stimulation of temporal tip produced slight fall, whereas variable effects were produced from other regions.

(ii) *Metabolic changes* .—Changes were observed in blood glucose, blood sodium and blood glutathione (reduced) levels but these could not be specifically related to the different regions of the limbic system. Changes in blood proteins and albumen : glogulin ratio were also observed.

(4) *Regulation of Endocrinal activities :*

(i) *ACTH secretion:*

After localisation of hypothalamic regions which regulate ACTH secretion, studies are now being conducted to find out whether this is mediated through pituitary stalk or through the autonomic nervous system.

(ii) *Thyrotropic hormone :*

Hypothalamic regulation of thyrotropic hormone is being studied by stimulating and ablating the different hypothalamic regions and then studying its effects on thyroid activity by histological studies and the uptake of labelled iodine.

(5) *Nervous regulation of the activity of the liver:*

(i) Investigations were carried out in collaboration with the Malaria Institute of India on the mechanism of production of necrosis of the liver under heavy infections by P. Knowlesi. In 13 monkeys, having restricted bilateral lesions in antero-medical part of middle portion of hypothalamus, heavy malarial infections did not produce any necrosis of the liver, whereas lesions in other hypothalamic regions could not prevent this necrosis. This indicates the regulating influence of hypothalamic regions of brain on the activity of liver. This influence may be operating through the vascular supply of the liver or by some other mechanism.

(ii) Liver-function studies have, therefore, been undertaken in all the animals having stimulations or ablations of the hypothalamic or the limbic lobe structures.

(6) *Physiological connections between different regions of the limbic system by evoked potential studies :*

(i) Such studies have demonstrated marked inter connections between different regions of the limbic system.

(ii) Vagal afferents have been shown to project to orbital surface of the frontal lobes and to certain paleocerebellar regions.

(iii) Physiological connections between reticular system of brain stem and limbic structures are being studied.

(7) *Effects of hypothermia :*

Hypothermia was produced in 41 dogs by the surface cooling method. Hypothermia alone, to as low a rectal temperature as 80°C., did not produce any fibrillation in heart. On the other hand, temperatures between 18°C. and 35°C. could produce ventricular fibrillation when left ventricle was simultaneously stimulated either mechanically or electrically. Acetylcholine and glutathione contents of heart were found to be higher in fibrillating group as compared to non-fibrillating group. This difference might be due to the fact that the average temperature of the latter group was 7°C. lower than the former group.

(8) *Mechanism of action of Reserpine and Largactil:*

Activity of different hypothalamic and limbic regions is being studied electro-encephalographically before and after injecting either of these drugs. Activity of hippocampus is occasionally increased under the effect of Reserpine.

II. Studies on Physiological changes in Yogs

These studies were started in May 1958. They have been planned on the following three lines :—

(i) In a large number of individuals undergoing yogic exercises at different 'yogashrams' in Delhi, studies have been undertaken to find out if such exercises improve the efficiency of working of the stems of the body.

(ii) Studies of higher nervous activity which may be produced during the stage of deep meditation in those yogis who have been practising it for a long time.

(iii) Studies to find out whether some of these yogis can develop voluntary control over the autonomic functions of the body particularly the voluntary stopping of the heart and the pulse.

In addition to the eletro-encephalographic investigation studies are carried out on the cardiovascular activity, respiratory function and exchange of gases, gastrointestinal activity, responses mediated through the autonomic nervous system, blood chemistry, etc.

So far 32 persons from different yogashrams have been investigated. In some of them the investigations have been repeated a second time. In addition, some persons not following any yogic practices have been studied to serve as controls. These investigations are still in progress.

199. Enquiry on studies on the growth of resistance in microorganisms against antibiotics under Dr. S. K. Bose at the University College of Science and Technology, Calcutta.

(1) The behaviour of sensitive parent and penicillin resistant variant (isolated at a level of 8000 units/ml) of the organism was studied to find out the difference in their serological reactions.

(2) It is observed that the given strain, as also its resistant variant possess two antigens—one somatic and another cell wall antigen.

(3) Induction of penicillin resistance does not seem to affect the cell-wall antigen to the same extent as it does the cell body or somatic antigen of *the organism which is rendered highly antigenic*. This may indicate that the seat of major change lies not on the surface but somewhere below the surface of a resistant variant.

(4) Resistant variant being *highly antigenic leads to the* production of high titre serum which may prove useful in treating cases of infection by penicillin resistant organisms.

200. Enquiry on the role of electrolyte imbalance in hypertension under Dr. Inderjit Singh at the S. N. Medical College, Agra.

There has been a controversy on the mechanism of energy release for muscular contraction. While it is commonly believed that breakdown of adenosinetriphosphate (ATP) releases the necessary energy for muscular contraction, evidence is also forthcoming that there might be another source for this energy.

While attempting to study the effect of ATP on unstriated muscle of frog's stomach, it was noted that after the usual treatment with 50 per cent glycerine and refrigeration, it did not respond to ATP with any contraction. Since this muscle did not lose its usual physiological responses, it should be expected to respond to ATP like other muscle models, if the latter substance was the primary source of energy for muscular contraction. In the present study, therefore, a detailed investigation of the effect of ATP on models prepared from this muscle has been undertaken.

These experiments were performed on transverse pieces of the stomach muscle of the frog, *Rana Tigrina*. After dissection, they were immersed in 50 per cent glycerine and stored in refrigerator at −15° to −20°C. for variable periods ranging from 24 hours to 6 months. Some muscles were stored at 0°C. The fluid was exchanged twice for fresh, precooled 50 per cent glycerol at daily intervals before storage. Before use, the fibres were placed in 15 per cent glycerol at 0°C. for one hour ; but 15 per cent glycerol caused marked lengthening of the muscle, and hence was considered unsuitable.

The effect of ATP was tested on loaded as well as unloaded pieces ; the latter were used to test for active relaxation, if any. The experiments were performed at room temperature, 30°C.

As reported previously, the effect of ions on frog's stomach muscle which has been refrigerated in 50 per cent glycerine is different from that on striated muscle. Potassium chloride causes contraction of glycerine-soaked striated muscle. Similarly treated frog's stomach muscle from the cardiac end actively relaxes to an extent of about 60 per cent in isotonic potassium chloride. Calcium or magnesium (0.01 M) antagonises this action, reducing the relaxation to 40 per cent.

In the present experiments, pieces of muscle were removed from the refrigerator and glycerine was removed by washing with potassium chloride solution, with or without magnesium. Treatment with potassium chloride solution was continued till a constant length was reached in the freely floating condition. To test the effect of ATP on loaded muscles, they were suspended in a bath ; to test for active relaxation, the preliminary solution was changed for that containing ATP with the muscle freely floating in a Petri-dish.

Effect of ATP on contraction.—The effect of sodium salt of ATP in concentrations of 1.4, 1.0, 0.5, 0.25, 0.1 and 0.05 per cent was tested. The muscles were treated beforehand with 0.112 M potassium chloride

solution either alone or with added magnesium chloride in concentrations of 0.001 to 0.01 M. In some experiments, calcium chloride in concentration of 0.05 and 0.01 M was also included with or without magnesium in the potassium chloride solution. The ATP was dissolved in the preliminary solutoin with which the muscle was treated but in a concentraction of 1.5 per cent. It was also used dissolved in distilled water. In over 50 experiments with loaded muscles, no contraction was ever observed. The muscles were stored in the refrigerator for 24–48 hours, 10 days, 1 month, 3 months and 6 months. In a number of experiments with unloaded muscles stored for varying periods ranging from 24 hours to 6 months no contraction was ever observed. The addition of creatinephosphate to ATP solution made no difference.

Effect of ATP on active relaxation.—In the above 100 experiments with unloaded muscles, 12 pieces relaxed actively to an extent of about 10–40 per cent. In muscles stored for longer time, no active relaxation was ever observed. As the positive results are very few. these findings are of doubtful value. But it is also possible that the failure to get active relaxation by ATP in a great majority of experiments might be due to preliminary active relaxation by potassium chloride, so that ATP was unable to cause any further relaxation.

It appears that ATP does not cause contraction of glycerinated unstriated muscle from the stomach of the frog *Rana tigrina*. These experiments thus throw doubt on the role of ATP as the primary source of energy in muscular contraction.

There is some possibility of active relaxation being produced by ATP. In this muscle, relaxation is attended with increase in oxygen consumption and lactic acid production which is abolished by iodoacetic acid and cyanide. Increased production of lactic acid during relaxation has been found by Mohme-Lundholm ; adrenaline, noradrenaline, isopropylnoradrenaline and ephedrine have a relaxing as well as lactic acid forming effect. The degree of relaxation tends to run parallel with the increase in lactic acid concentraction. Excess of calcium ions inhibits both the relaxing and the lactic acid-forming effects of adrenaline.

There is thus no doubt that there is a breakdown of glycogen during relaxation of unstriated muscle, and as breakdown of glycogen is known to be preceded by a breakdown of phosphocreatine and ATP, the production of active relaxation by ATP is theoretically possible ; and hence the positive results in the actual experiment though appear to be significant,

201. Enquiry on screening of penicillin compounds under Dr. P. D. Kulkarni at the Research Laboratories of the Hindustan Antibiotics, Pimpri.

As stated in last year's report, compounds of penicillin are taken for investigation with a view to find out if there is any correlation between the maintenance of serum concentrations and the molecular structure of the compound. The different series investigated are :

(a) N-N Dialkyl Ethylene Diamines by introducing groups of homologous series from C2 to C-12.

(b) N-N Diaryl Diamines by substitution in benzene nucleus.

(c) Steroid compounds.

(d) N-Alkyl N'—Benzyl Ethylene Diamine series.

Preliminary studies of these compounds include :

(1) *Potency*: (a) By microbiological assay using B.subtilis as test organism, and

(b) Iodimetric method.

(2) *Stability*: In artificial gastric juice (pH : 4.5) :—Suspensions of the salt were prepared in buffer pH 7 (control) and also in artificial gastric juice. Both were incubated at 37°C. for 2 hours., and bioassay was done.

(3) *Toxicity*: Tested in mice (weighing 25 g.) by intraperitoneal injection of 1000 units suspended in 1 c.c. normal saline. (0.1 ml. of Tween 80 was as suspending agent.)

(4) *Blood Serum Concentrations :*

(A) *After Intramuscular Dose* .—Suspensions containing 800,000 units prepared in total vol. of 2 ml. (sterile distilled water, 1.5 cc. and 0.5 ml. of 4 per cent gum acacia as suspending agent) was injected intramuscularly into rabbits weighing between 2.0 and 2.5 kg. Two or more rabbits were used for each salt. Rabbits were bled at intervals of 24, 48, 72 and 96 hours,, and serum concentrations of penicillin were estimated serially till the blood serum level fell below 0.$\mu\frac{1}{2}$ u/ml.

(B) *After oral Dose* : For oral feeding a simple wooden mouth gag was used. The compound was administered orally through a sterilized rubber catheter.

INFERENCE :

The compounds from steroid series compare well with D.B.E.D. sometimes even better results were obtained with the former. They

appear to show a tendency towards an increase in tissue concentration which is now being investigated further. 'Octo 1' compound from the 1st series was found to keep blood level in rabbits for over 5 days. It is being investigated on human volunteers.

202. Enquiry to study the diuretic and antidiuretic Ayurvedic remedies under Drs. O. C. Gulati, B.M. Mazumdar and G. K. Karandikar at the Medical College, Baroda.

Extracts of Punarnava, Gokhru and liquid extract of Punarnava (I.P.) produced diuresis in rats and in anaesthetized (Pentobarbitone sodium 30 mg./kg. i.p.) female dogs.

The aqueous extracts of Gokhru and Punarnava contain 60 per cent and 65 per cent respectively of potassium expressed as potassium chloride. Since the plant extracts contain high amounts of potassium, it was considered desirable to compare the diuretic activity of the extracts with equivalent amount of potassium. It was found that the entire diuretic activity of the plant extracts was due to their potassium content, hence further plan to study the mechanism of diuresis was given up. The results are summarized in Tables I & II.

TABLE I.

Mean diuretic activity and the mean chloride content of the urine samples in rats :—

Sr. No.	Name of the substance administered with dose and route of administration.	Mean diuretic activity with standard error.	Mean chloride content with standard error.
1.	Water extract of Punarnava 1g./kg. orally.	0.681 ± 0.0596	0.940 ± 0.0782
2.	Liq. ext. of Punarnava (I. P. '55) 2.56 ml/kg. orally.	0.639 ± 0.122	0.662 ± 0.752
3.	Water extract of Gokhru 1.2g./kg. orally.	0.860 ± 0.153	1.145 ± 0.0704
4.	Urea 960 mg./kg. orally.	1.00	0.756 ± 0.0633
5.	Potassium chloride.	1.544	1.143

TABLE II.

Mean urinary excretion and mean chloride content of urine samples in dogs.

Sr. No.	Name of the substance administered with dose and oute of administration	Mean diuretic activity with standard error.	Mean chloride content with standard error.
1.	Nasal.	12.05 ± 3.06	0.325 ± 0.191
2.	Urea 1·0g./kg. orally	22.7 ± 5.13	0.115 ± 0.013
3.	Water extract of Punarnava 0.5 g./kg. orally.	24.2 ± 2.33	0.315 ± 0.139
4.	Water extract of Gokhru 0.5 g./kg. orally.	30.8 ± 6.94	0.122 ± 0.023
5.	Potassium chloride 0.3 g./kg. orally.	26.6 ± 2.77	0.749 ± 0.028

203. Studies on transmethylation of nor-adrenaline to adrenaline in relation to cardiac arrythmias under Dr. B. C. Bose at the Medical College, Indore.

The object of the work was to study the process of transmethylation of nor-adrenaline to adrenaline in heart and liver, and to find out the effect of antifibrillatory drugs on this transmethylation process.

A series of *in vitro* investigations were carried out in heart and liver tissues of rats using choline, methionine and acetylcholine as methyl donors. The samples were incubated for 4 to 24 hours at 37°C and the contents of adienaline and not-adrenaline in control as well as test samples were estimated by the modified method of Von Euler (1949) using iodine as the oxidising agent. It was observed that in heart, transmethylation with choline, methionine and acetylcholine occurred to the extent of 2 per cent, 2.8 per cent and 7.8 per cent respectively, in 12 hours, and 3.8 per cent, 6.8 per cent and 10.2 per cent respectively, in 24 hours. In liver, maximum transmethylation of 7.8 per cent and 10.2 per cent in 12 and 24 hours respectively, was seen when acetylcholine was used as methyl donor. The other methylating agents did not produce any significant effect.

A series of experiments were performed to study the effect of vitamin B_{12} and folic acid on the mechanism of transmethylation process of nor-adrenaline to adrenaline in liver and heart tissues of rats. On incubating the samples of liver and heart with either folic acid or vitamin B_{12} with or without added choline and methionine it was observed that addition of B_{12} to choline increased the methylation from 4.2 to 9.7 per cent, whereas folic acid increased the same from 2.05 to 5.7 per cent only in heart tissue.

To study the process of demethylation of adrenaline to nor-adrenaline in heart and liver tissues of rats, the samples were incubated with ethanolamine, adrenaline and ATP for 12 and 24 hours. The observations indicated no change in the content of adrenaline showing thereby that probably ethanolamine did not take part in the process of demethylation. Further work on the effect of other demethylating agents in the conversion of adrenaline to nor-adrenaline is in progress.

204. Enquiry on the effect of rutin and related glycosides on the carbohydrate metabolism of liver under Dr. Mohan Singh and Shri Amar Singh at the Medical College, Amritsar.

The enquiry was confined to the effect of hesperidine-methyl chalcone on glycogenolysis in rat liver.

A well fed rat is killed by cervical dislocation, and abdomen opened. The liver is excised immediately and put on crushed ice. Thin slices are cut from small piece of the liver and kept in Krebs' saline cooled on crushed ice. A slice is blotted with filter paper and transferred to a cold Warburg flask containing 1.2 ml. of Krebs' saline. To one such flask is added 1.mg./ml. hesperidine methyl chalcone. The flasks are fitted on to manometers and the manometers transferred to Warburg apparatus. They are incubated at 37°C. for a period of 45 minutes, and shaken at 100 oscillations per minute. The flasks are removed after 45 minutes the tissue removed from the flask, washed in distilled water, blotted and transferred to a small tared weighing bottle It is dried overnight at 110°C. and weighed. A measured aliquot of the fluid in the flask is diluted appropriately and its glucose content determined by the method of Folin and malmros. Glucose equivalent of the hesperidine methyl chalcone is determined in the solution used, and substracted from the values for test flasks. Glycogenolysis is calculated as mgm. of glucose produced in one hour by 1 mg. of dry tissue.

Eighteen sets of experiments were performed. The results were analyzed statistically by two methods. By both the methods, the inhibition of glycogenolysis by hesperidine methyl chalcone is significant.

205. Enquiry on histamine content and histaminolytic properties of cerebrospinal fluid of animals and humans in health and disease under Dr. P. K. Kar at the S.C.B. Medical College,

Qualitative and quantitative tests for the histaminolytic property of cerebrospinal fluid were conducted by incubating a known amount of histamine with the fluid and then estimating the reduction of the histamine content. Bio-assay of histamine was carried out on guinea pigs ileum- A steady reduction in the histamino content was observed and the rate of histamine destruction per ml. of cerebrospinal fluid per hour was calculated.

2. The same procedure was repeated with dogs' cereprospinal fluid. In this case, the histaminotytic action was found to be slow and delayed compared to human samples.

3. The concentrated solution of the products of dessication were tested for spasmogenic action on the guinea pig's ileum. The mild spasmogenic property observed could be partly blocked by a dilute solution of Anthisan and further blocked by a solution of Atropine.

206. Studies on vascular reflexes in spinal and non-spinal preparations under Dr. S. R. Mukherjee at the Medical College, Calcutta.

I. Distension of small segments of jejunum or ileum in urethane-anaesthetised cats causes an appreciable rise of arterial blokd pressure.

2. When the buffer nervex (vegi and carotid sinus nerves) are intact in non-spinal preparations, the rise in blood pressure is slight, but considerable rise in blood pressure occurs when the influence of these buffer nerves is abolished.

3, The rise in blood pressure is influenced by intraluminal tension and intraluminal fluid volume, therefore, the effective stimulus appears to be the tension and stretch of the intestinal walls.

4. Reflex vascular responses similar to those obtained in non-special preparations can be satisfactorily elicited in acute spinal in animals by distending the intestinal lumen without being associated with the viscero-somatomotor reflexes. The rise of blood pressure in spinal preparations is more pronounced than that in non-spinal preparations, provided the basal blood pressure in the recovery stage after spinal shock, is moderately high.

5. Preliminary investigations on the respective role of sinus nerves and vagi in this homeostasis indicate that sinus nerves play a more important role.

207. Enquiry on participation of parathyroid glands in acute systemic stress under Dr. T. H. Rindani at the Topiwala National Medical College, Bombay.

It is well known that profound metabolic changes occur in animals during adaptation to stress. The two important systems of the body, *viz*: the nervous and the endocrinal, are directly responsible for these changes which are aimed at homeostasis.

A lot of work has been done to study the role of the hypophysis-adrenocortical axis as well as that of the adrenal medulla in relation to systemic stress but in spite of changes in calcium and phosphorus metabolism observed in this condition no systematic study of the participation of the parathyroids seems to have been undertaken.

The present enquiry was, therefore, undertaken to evaluate the role, if any, of the parathyroid in stress.

So far, effects of acute systemic stress (formalin injections) on the urinary excretion of inorganic phosphorus in normal and parathyrodectomised rats were studied to see if the phosphaturia of stress continued to occur in the absence of the parathyroids. It was observed that this increase in the phosphaturia was not entirely prevented by the absence of the parathyroids in animals.

In order to assess the activity of the endocrine system in stress, estimation of 24 hour excretion of 17 hydroxycorticosteroids in urine of the animals was considered necessary. For this purpose, normal values for the excretion of these steroids had to be established in the animals used in this laboratory. Accordingly, 42 observations on 7 groups of animals (4 in each group) were carried out. It was observed that the average daily excretion of the steroids under normal physiological state was 33 μg.

208. Investigations on gastric secretory function by tubeless method as compared to the standard intubation method under Dr. M.L. Pai at the Medical College, Baroda.

The examination of gastric secretory function is carried out as a routine by the method of fractional gastric analysis. This method however, has some disadvantages. Many of the subjects usually do not like the introduction of the stomach tube. Secondly the presence of the tube is likely to alter the composition of the juice. A tubeless method, therefore, will have no such disadvantages. Use of a cation exchange indicator has been suggested for such a test. Diagnex is a product obtained by replacing the hydrogen ions of the carboxylic acid group with quininium ions. In the presence of free hydrochloric acid, e.g. of gastric juice, its hydrogen cation will replace and release the quininium cation, which is absorbed in the small intestine, and approximately one-third of it is excreted in the urine. The test is thus based on the quantitative estimation of quinine in urine.

Persons showing achlorhydria, hypochlorhydria, isochlorhydria or hyper chlorhydria, as determined by alcohol meal test and histamine test, (Pai, 1957) were studied by the diagnex test which was already standardized (Pai, 1956). Total two-hour urinary excretion of quinine was determined fluorophotometrically after the ingestion of the cation exchange indicator. The original fluorescence in the control sample of urine was deducted from the total fluorescence obtained in the test samples of urine. The excretion of quinine was expressed in mcg. per two-hour total urinary excretion. The average highest free acidity of the above four groups of persons was compared with the quinine excretion. Sixty-two persons were studied before treatment and nine persons after treatment thus making up a total of seventy-one subjects.

A statistically significant direct relationship between the highest free acidity obtained by the fractional method and the urinary quinine excretion after the tubeless method was observed. (Pai in press, I. J. M. R. Sept. 1960).

209. Studies on 17-Ketosteroid output in health and disease of Indians under Dr. J. C. Sachdev at the M.G.M. Medical College, Indore.

17-Ketosteroid output was estimated in 100 normal healthy subjects. The values ranged from 7.3 mg. to 27.4 mg. per 24 hours with an average of 13.8 mg. The values in females ranged from 6.0 to 21.4 mg. per 24 hours with an average of 12.1 mg. No appreciable difference in the excretion of 17-Ketosteroids was observed between the age groups of 17 to 40 years, but a slight decrease was noticed with increase in age above 50 years. The above results are in agreement with Friedmen's observation that 17-Ketosteroid output in normal Indian subjects is in general lower than that reported for normal healthy Europeans. An analysis of the results in terms of dietetic habits indicated that there was no appreciable difference in the output of 17-Ketosteroids in vegetarian and non-vegetarians.

Corticosteroids in urine were estimated by the method of Heard, Sobel and Venning. The values ranged from 1.02 to 1.19 mg, per 24 hours with an average of 1.09 mg. There was a direct relationship in the excretion levels of corticosteroids and 17-Ketosteroids.

17-Ketosteroids were also estimated in about 20 cases suffering from Para-sprue syndrome. The average value in this condition was 7.0 mg. per 24 hours (with a range of 6.4 to 8.2 mg.) which was considerably lower than that observed in the normals.

Similar work was carried out in alloxan-diabetic rats. It was observed that the induction of alloxan diabetes was associated with a decrease in 17-Ketosteroid output. The normal value in rats was 0.73 mg. per 24 hours. In diabetic rats the value was reduced, ranging from 0.11 mg. to 0.3 mg. with an average of 0.15 mg. per 24 hours.

210. Drug Research Unit at the Haffkine Institute, Bombay.

The drug Research Unit started functioning from 15 Februry 1958. Two plants, *Withania Somnifera* and *Coleus aromaticus*, were taken up for investigation.

A systematic chemical investigation of the roots of *Withania somnifera* was carried out. Presence of alkaloids, fat, phytosterols, sugars, resin, and inorganic substances, was confirmed. The total alkaloids were obtained by extracting 50 kg. of the roots with 60 per cent alcohol. Qualitative analysis by paper chromatography revealed the presence of at least six different alkaloids. Work on the isolation of each of the alkaloids and other non-alkaloidal principles is in progress.

The juice of the leaf of *Coleus aromaticus* was tested *in vitro* for anti-bacterial properties, and *in vivo* in experimental cholera. Neither the juice nor the volatile principles from the leaves showed any anti-bacterial or curative properties in experimental cholera.

A sample of "Athomin" claimed to be useful in the treatment of cholera, was tested for its curative action in experimental cholera. The sample, however, failed to show any curative or preventive action in cholera, when tested in infant rabbits.

211. Drug Research Unit at the R.G. Kar Medical College, Calcutta.

I. Barks of 7 species of Terminalia (T. arjuna, T. tomentosa, T. bialata, T. belorica, T. catappa, T. chebula, T. paniculata.) have been studied for botanical characters. Preliminary chemical and pharmacological observations have been made with :

1.1 Cold aqueous and alcoholic (60 per cent) extracts (1 in 10) of the barks of 7 species of Terminalia.

1.2 Inorganic salt free extracts of *T. arjuna* and *T. tomentosa*.

1.3 Fractions TAw_1, TAw_3 and TAa_1 of *T. arjuna*.

2. A more detailed pharmacology of the aqueous extract of *T. tomentosa*.

3. Crude and inorganic salt free aqueous extracts of both *T. arjuna* and *T. tomentosa* gave positive tests for taurins, saponins, fehling's-solution-reducing bodies and for substances precipitable by alkaloidal reagents.

4. Aqueous extract of *T. arjuna* was extracted successively with ethyl acetate (fraction TAw_1), chloroform (fraction TAw_2) and acetone (fraction TAw_3,)

4.1 Fraction TAw_1 showed presence of tannis only.

4.2 Fraction TAw_2 were not tested because of small yield.

4.3 Fraction TAw_3 did not give positive test for any of the principles noted in para 3.

5. Alcoholic extract of T. *arjuna* was treated with activated charcoal and filtered.

5.1 The dry residue (TAa_1) obtained after evaporation of the filtrate gave positive tests for tannins and fehling's-solution-reducing substances,

5.2 Successive extractions of the treated charcoal with ethyl acetate and other gave solid residues (TAa_2 and TAa_3 respectively) on evaporation of solvents which could not be tested due to small yields.

6. Preliminary pharmacological studies were carried out with the extracts and fractions mentioned in para 1. Both watery and alcoholic extracts of all the seven species of Terminalia examined gave a positive inotropic effect but only four of them,- viz., *T. belerica*, *T. arjuna*, *T. catappa* and *T. tomentosa*,

showed positive chronotropic effects, the others did not affect the heart rate. There was no significant difference between the watery and alcoholic extracts so far the chronotropic changes are concerned. With regard to the inotropic effects, however, the alcoholic extracts of all the species except *T. tomentosa* were more potent than the corresponding watery extracts. Measured in terms of percentage increase in the amplitude of contraction of frog's heart, watery extract of *T. tomentosa* was 2.5 times more potent than the corresponding alcoholic extract.

6.1 Small number of observations carried out with inorganic salt-free extracts of T. arjuna and T. tomentosa and fractions TAw_1 & TAa_1 gave results similar to those obtained with crude parent extracts.

6 2 Fraction TAw_3 when tested on frog's heart was inactive.

7. Further studies with the aqueous extract of *T. tomentosa* demonstrated its potent pharmacodynamic activity. Extract was freshly made every 15 days and stored at 3°C.

7.1 When tested on the hearts of toads and cats (*in situ*) marked augmentation of rate and amplitude of contraction was noted in moderate doses. Progressive slowing, A-V block and complete arrest of the heart occurred at higher dosage.

7.2 Hypotensive effect of short duration (5 min.) was noted in cats with dosages that stimulated cardiac activity (1 ml. of 1 : 10 dilution of the extract, I. V.); with increasing doses 1 ml. of 1:5 to 1:2) the hypotension became progressively persistent and profound commensurate with deterioration of cardiac activity. Up to a limit, both hypotensive effect and cardiac depression could be annulled by Adrenaline.

7.3 Respiration (cat), unaffected by small doses decreased in amplitude and rate at a dose that caused hypotensive effect and cardiac stimulation (1 ml. of 1:10); increasing dosage produced progressive deterioration of respiration ending in complete failure at higher dose levels (1 ml. of 1:5 to 1:2).

7.4 An increase in the tone and amplitude of contraction of isolated intestine (Guineapig) was noted at concentration of 10^{-3}, preceded by a short period of relaxation.

7.5 No irritant or anaesthetic effects were noted when the freshly prepared crude extract was applied to the eyes or skin of rats, rabbits or cats.

7.6 The crude extract (injected in doses of 0.2 to 0.5 ml. per 50 g./B.W.) caused systolic arrest of toad's heart and immediate death in white mice.

8. The investigation reported herein, is essentially exploratory in nature. The findings, however, emphasize the need for, re-examination of older data. While all the seven species studied showed cardiac activity, Cains and co-workers found *T. belerica* and *T. arjuna* to be merely mildly diuretic. of the 15 commoner Indian species of Terminalia examined by these workers, only *T. arjuna* has been studied by other workers (Roychoudhurf *et al*). Current findings indicate that attention to *T. arjuna* has been too exclusive, and that investigations of other species, particularly *T. tomentosa*, may be more fruitful.

212. Drug Research Unit under Dr. M. L. Gujral at the K. G. Medical College, Lucknow.

The antiarthritic and the anti-inflammatory effect of *Glycyrrhiza glabra* (Mulethi) and the gum resin of *Balsamodendron mukhul* (Guggul) have been studied employing Brownlee's Formalin-induced arthritis and Seyle's Granuloma pouch technique in the rats.

Glycyrrhiza glabra.—The active principle of *glycyrrhiza glabra*, the diglucuromide glycyrrhizin and its hydrolytic product glycyrrhitic acid, were tested along with other extracts, using hydrocortisone and butazolidine as the reference standards. After statistical analysis of the data both glycyrrhizin and glycyrrhitic acid were shown to have an activity superior to the standard drugs employed. The work is further continued and the pharmacology and the toxic effects of the active principles of the drug are being investigated.

Guggul.—As with glycyrrhizin, preliminary reports on the antiarthritic and anti-inflammatory effects of the drug guggul were made in July 1957 at the meeting of the Drug Research Units held in Lucknow and in last year's I.C.M.R report. The resin fraction of the oleogum was found to be responsible for the antiarthritic and the anti-inflammatory effects of the drug. Further fractionation of the resin has given acidic and ketonic substances. Toxicity studies with these substances are in progress.

213. Drug Research Unit under Dr. G. S. Raghunath Rao at the Medical College, Mysore.

Since 1st February, 1959, the following plants have been collected and identified; (1) *Boerhaavia Repens*, (2) *Mimosa Pudica* (3) *Nerium Odorum* and (4) *Thevetia Nerifolia*.

Preliminary trial for the extraction of crude and active principles from *Atropa belladonna* and *Digitalis purpurea* have been completed. Techniques for the isolation of animal tissues for the trial of the various drugs are being tried. Apparatus for the conduct of experiment on diuretics has been set up.

214. Drug Research Unit under Dr. G. Achari, P. W. Medical College, Patna.

The tuberous roots of Amorphallus campanulatus, a perennial stemless herb cultivated throughout India, has been chosen for an investigation of its pharmacological actions.

The tuber was dried in the sun and powdered. Weighed amount of the dry powder was extracted with hot water. The aqueous extract was ten times concentrated after filtration and a study of the pharmacological action was undertaken with this product.

1. *Action on frog's heart :*

A striking reduction in the amplitude of contraction of the heart was noticed. On increasing the dose the heart stopped in systole for a few seconds and started contracting automatically. Atropine had no effect on the above action. The extract did not block the action of adrenaline on the heart.

2. *Action on blood pressure and respiration of dog:*

When injected intravenously a considerable fall in blood pressure with hurried respirations was noticed. Antihistamines had no effect on the action of the extract on blood pressure.

3. *Action on rat uterus :*

When added to the bath, the extract produced a marked increase in the force of contraction and the tone of the uterine musculature.

4. *Action on the ileum of guinea pig :*

When added to the bath the extract produced a marked increase in the force of contraction and the tone of the gut.

Further work is in progress.

215. Drug Research Unit under Dr. Ranita Aiman at the B.J. Medical College, Poona.

Drugs Investigated

The powdered barks of *Ficus Bengalensis* and *Ficus glomerate* were used as 10 per cent water and alcoholic extracts for oral administration. Some portions were evaporated and solubilised in the usual manner for intravenous studies. The juice of the pulp of fresh fruits of *Eugenia gambolana* was used orally.

Object

The object was to determine if these drugs exert any action on the fasting blood sugar (F.B.S.) of normal animals or animals made diabetic by alloxan or pancreatectomy. The studies were carried out on rabbits and dogs.

Method

Estimations of FBS were carried out on five normal and two alloxan treated robbits over a period of five hours; five normal, one depancreatised and four alloxan treated dogs over a period of four hours. These were compared with the results of distilled water administered in a similar manner.

Results

I. Ficus Bengalensis :

Oral administration of water extract resulted in a maximum fall of 22 per cent in the blood sugar level with a dose of 2.5 g. in normal rabbits, and 18 per cent in normal dogs with a dose of 10 g. The respective figures with distilled water were 2 per cent and 9 per cent. The alcohol extract of 5g. gave a 7 per cent fall in rabbits compared to 18 per cent fall in the controls.

Intravenous water extract led to a rise in blood sugar in rabbits accompanied by a depression of the central nervous system. In dogs intravenous alcohol extract of 20 g. gave no appreciable fall. Similarly, no appreciable fall in the blood sugar was obtained either in the depancreatised dog or in alloxan treated rabbits given water extract orally or in alloxan treated dogs given the water and alcohol extract orally.

II. *Ficus Glomerata* :

Administration of water extracts orally resulted in a maximum fall of 20 per cent with dose of 2.5 g in normal rabbits and 20 per cent in normal dogs with a dose of 10g. compared to a fall of 18 per cent and 9 per cent respectively with distilled water. Alcohol extract of 5 g. in normal rabbits gave a 9 per cent fall, compared to 18 per cent in the control,

Intravenous administration of alcohol extract of 15 g. gave a 10 per cent fall in normal dogs.

In one depancreatised dog and in alloxan treated rabbits oral water extract gave no significant fall in blood sugar. Similarly in alloxan treated dogs oral water extract and oral and intravenous alcohol extract gave no appreciable results.

III. *Eugenia Jambolana :*

In normal rabbits the water extract of 50 g. of pulp produced a 30 per cent fall in four hours preceded by a 45 per cent rise in one hour as compared to 18 per cent fall and 70 per cent rise during the corresponding periods with distilled water. Other studies in alloxan treated rabbits and dogs, and normal dogs gave no appreciable results.

Chemical studies on Ficus Bengalensis and Ficus Glomerata

Quantitative analyis for moisture, ash, organic matter,acid insoluble ash, water soluble matter, calcium, potassium, magnesium crude fibre colouring matter, woodgums, sterols, water soluble tannins and hydrocarbons was carried out.

Qualitative analysis for succinic, phthalic and oxalic acids, resins xanthoproteins, and alkaloid-like substances of indole structure was also carried out.

Fractional analysis on the basis of solubility in petroleum, ether ether, chloroform, ethyl acetate, alcohol and benzene was completed.

216. Drug Research Unit under Dr. C. L. Malhotra at the Lady Hardinge Medical College, New Delhi.

The work on *Herpestis monniera*, *Withania ashwagandha*, and *Rauwolfia* alkaloids was continued, and preliminary studies were started on *Acorus calamus*.

A. Herpestis Monniera.

1. *Chemical Studies :*

A non-active non-reducing sugar, d-mannitol, and a glycoside-saponin principle were isolated in pure form, and the melting point and physical properties of these substances were reported previously. This year further chemical studies with *Herpestis monniera* and its fractions were carried out.

The percentage yield of d-mannitol and glycoside-saponin principle was 0.8 per cent and 0.02 per cent respectively.

Chemical investigations of the glycoside-saponin principle were carried out with a view to establish the molecular structure of the compound. Though the Liebermann—Burchard test was positive, the various colour reactions for the steroidal ring were neither specific nor was there any marked colour change. The reaction and Kellar-Killiani tests were negative showing the absence of unsaturated lactone ring and desoxysugar respectively. It was insoluble in hot dilute alkali indicating the absence of lactone ring. Tests showed the presence of reducing nuclei in the molecule. It did not contain nitrogens, sulphur phosphorus and halogens. The glycoside-saponin principle contained 50.8 per cent carbon, 41.3 per cent oxygen and 7.9 per cent hydrogen. The empirical formula is suggested as $C_{10}H_{18}O_6$.

Herpestis monniera was found to contain in addition, three potassium salts—chloride, sulphate and nitrate, and the presence of alkaloid in a fraction was confirmed. The alkaloid has not yet been isolated in pure crystalline form.

2. *Pharmacological studies (of glycoside-saponin principle):*

(i) *Cardio-vascular system :*

(a) *Frogs :*

The cardiotonic action of glycoside-saponin principle on frog's heart by single injection and also by continuous perfusion, were confirmed by experiments on Straub's ventricle and on hypodynamic frog's heart. The hypodynamic heart was produced by either raising the venous pressure or by perfusing with Ringer's solution containing 1/6 of normal calcium. In such preparations the glycoside-saponin principle in doses of 1 to 4 mg. showed pronounced cardiotonic action as indicated by marked positive inotropic action and marked increase in cardiac output.

(b) *Dogs :*

In anaesthetised dogs the principle had no significant effect on the blood pressure, heart rate and respiration in doses of 10 mg./kg. body weight.

(ii) *Central nervous system :*

(a) *Albino rats*

Sedative and hypnotic actions.—In doses of 1.0 mg/100g. body weight intraperitoneally it had a sedative effect in 75 per cent of albino rats. In doses of 1.5 mg./100g. body weight sedative effect was seen in all the rats. A dose of 2.0 mg./100g. body weight produced hypnotic effect as judged by the absence of righting reflex.

Analgesic action.—Analgesic effect in albino rats was tested by the rat tail method. In doses of 2.0 mg./100g. body weight it had analgesic effect in 50 per cent of the rats.*

Anticonvulsant action.—It did not show any anticonvulsant action against metrazol-induced convulsions.

(b) *Guinea pigs :*

Glycoside-saponin principle was administered intraperitoneally and intravenously in unanaesthetised guinea pigs. In doses of 10 to 20 mg./kg. body weight the animal showed neurological involvement as evidenced by lack of voluntary movement, diminished muscular tone of limbs, lack of equilibrium and delayed righting reflex. With doses of 40 mg./kg. body weight intravenously (given at 2 mg./kg./min.) the animal was completely anaesthetised for a period of 2½ hours after which there was partial recovery. 25 mg./kg. body weight was lethal to all the guinea pigs tested, but the animals died 24 to 72 hours after the administration.

(iii) *Smooth muscle :*

(a) *Rabbits* .—On the isolated ileum of rabbit the glycoside-saponin principle, in concentrations of 1 : 20,000 and 1 : 10,000, had a marked spasmodic action. The onset of action, however, was delayed by about 15 to 60 seconds.

(b) *Guinea pigs :*

The same spasmodic effect was seen on isolated guinea pig's ileum.

(c) *Albino rats :*

In concentrations of 1 : 10,000 and above it had a marked spasmodic effect on isolated rat's uterus. The onset of action was delayed by 15 to 60 seconds in the case of rats also.

(iv) *Toxicity :*

In albino rats LD50 by intraperitoneal route was found to be 2.5 mg./100g. body weight. Death usually occurred in 24 to 96 hours after injection. Macroscopic and microscopic examinations of tissues 24 and 48 hours after administration of 2.5 mg. of the drug/100 g. body weight showed haemorrhages in lungs and fatty degeneration of liver.

B. WITHANIA ASHWAGANDHA.

1. *Chemical studies :*

(i) *Roots :*

(a) Roots were extracted with various solvents. The percentage yield in each case and the presence of different chemical substances with different solvents are given below.

Solvent.	per cent yield of extract	Alkaloids	Glycoside	Reducing Sugars	Tannins
1. Petroleum ether, (40°–60°C)	0.45	—	—	—	—
2. Solvent ether	0.20	+	+	—	—
3. Chloroform	0.21	+	+	+	—
4. Alcohol	4.19	+	+	+	—

(b) *Dulcital* .—A white crystalline substance melting 185°C was isolated from the alcoholic extract, and it has been identified as dulcitol, a sugar.

(c) *Total alkaloids* .—The investigations with different solvents for the isolation of total alkaloids showed that the yield was maximum with acid extraction, which method was later used for the isolation of total alkaloids. The fractionation of the total alkaloids into different individual alkaloids in pure form is in progress.

(ii) *Leaves : (Withania Somnifera)*

(a) Leaves were extracted with different solvents. The percentage yield of each extract and the presence of different chemical substances with different solvents are given below.

Solvent	Per cent yield of extract	Alkaloids	Glycosides	Reducing Sugars	Tannins
1. Petroleum ether (40°–60°C)	1.32	—	—	—	—
2. Solvent ether.	1.02	—	—	—	—
3. Chloroform.	3.36	—	—	—	—
4. Alcohol.	10.44	+	+	+	+

(b) The alcoholic extract yielded a white compound, which gave positive tests for sterol.

(c) A large number of inorganic salts were isolated from the alcoholic extract.

2. *Pharmacological studies* :

The pharmacological actions of the crude total extract of *Withania ashwagandha* were reported previously. This year the total alkaloidal extract has been investigated and its activities are being reported. The total alkaloidal extract was insoluble in water. A 2 to 4 per cent solution was made in 50 per cent alcohol, and in every case proper controls with blank 50 per cent alcohol were kept.

(i) *Cardio-vascular system :*

(a) *Dog* :—In anaesthetised dogs total alkaloidal extract in doses of 10 to 40 mg./kg. body weight produced moderate to marked fall in blood pressure of acute onset and prolonged duration. There was a moderate degree of bradycardia. In "spinal" dogs it had no significant effect showing that the hypotensive effect was probably not due to direct action on the blood vessels.

It blocked the pressor response of nicotine, but the respiratory stimulating action of the latter was unaffected. It blocked the contraction of the nictitating membrane on stimulation through cervical sympathetic chain. It had no adrenolytic action. It also blocked the cardio-inhibitory effect of vagus but had no antiacetylcholine action. It was concluded that the total alkaloids have autonomic ganglion blocking action. These findings suggest that the hypotensive action is at least partly due to ganglionic blocking action.

(b) *Frogs* :

In perfused frog's heart the total alkaloids produced an immediate

transient cardiac depression followed by marked positive inotropic and positive chronotropic actions. The transient cardiac depression could not be prevented by atropinisation.

(ii) *Respiratory system :*

Dogs :

In anaesthetised dogs intravenous administration of 10 to 40 mg./kg. of total alkaloids produced moderate to marked stimulation of respiration The stimulation was more in the rate and less in the depth. The depth of respiration in many cases was actually reduced, but the rate doubled or even trebled.

(iii) *Skeletal muscles :*

Frogs .—The total alkaloids showed a spasmodic effect on frog's rectus muscle. The action was directly on the muscle as it could neither be antagonised nor prevented by neuromuscular blocking agents. The spasmodic effect was of slower onset and could not be easily abolished. It subsequently reduced the sensitivity of rectus muscle to acetylcholine.

(iv) *Smooth muscle :*

(a) *Rabbit* .—On isolated rabbit's ileum the total alkaloids had a marked relaxant effect.

(b) *Rats* .—On isolated rat's uterus the total alkaloids diminished amplitude of contractions.

(v) *Nervous system :*

The pharmacological studies of the total alkaloidal extract on nervous system are under progress. Preliminary studies in albino rats showed that the total alkaloidal extract, when administered intraperitonially as a suspension in 10 per cent gum acacia, causes sedation.

C. Rauwofia serpentina.

The work on this is being carried out in collaboration with Dr. B. K. Anand, Head of the Department of Physiology, All-India Institute of Medical Sciences, New Delhi, and Dr. Baldev Singh, Neurologist, Tirthram Shah Hospital and part-time Lecturer in Neurology, Lady Hardinge Medical College, New Delhi.

The following investigations were carried out with the pure alkaloids:

1. *Effect of reserpine on limbic system of cat :*

The electrodes have been implanted in the various areas of limbic system and E.E.G. recordings have been taken before and after reserpine to find out if it has any action on limbic system.

2. *Effect of reserpine on hypothalamus especially the 'feeding' and 'satiety' centres :*

The electrodes have been implanted in the hypothalamus of cats and E.E.G. recordings have been taken from the feeding and satiety centres to localise the effect of the drug on these centres. The work is in progress.

D. ACORUS CALAMUS.

The rhizomes of *Acorus calamus* were extracted with different solvents. The percentage yield and the different chemical constituents in each are given below.

Solvent	Per cent yield of extract	Alkaloids	Glycosides	Sugar	Tannins	Any other
Petroleum ether (40-60°C).	6·78	+	—	—	—	Oily liquid
Solvent ether.	0·58	+	+	—	—	Oily liquid
Chloroform.	0·36	+	—	—	—	Oily liquid
Alcohol.	7·79	+	+	+	—	Oily liquid

Further chemical analysis in detail is being done.

217. Enquiry on ascorbic acid and glutathione contents of blood in some infectious diseases under Dr. B. Chakrabarti, Department of Physiology Nilratan Sircar Medical College, Calcutta.

Fourteen cases of pyogenic meningitis, 2 cases of tuberculous meningitis, 4 cases of enteric fever, 1 case of smallpox, 7 cases of cholera, 21 cases of tetanus and 22 cases of non- infectious diseases were investigated for reduced ascorbic acid, dehydro-ascorbic acid and glutathione levels of blood in relation to the clinical condition and the treatment adopted. Only one sample of blood from each case was collected. Some were admitted to the hospital with previous treatment outside. Blood was drawn from a few cases (14 cases of tetanus, 6 cases of pyogenic meningitis, 2 cases of tuberculous meningitis and 6 cases of cholera) before any treatment. The results revealed that dehy-dro-ascorbic acid content was considerably increased with concomitant decrease of reduced glutathione and ascorbic acid during acute phase of the disease.

218. Studies on cardiac output at rest and on exercise in hyperkinetic states under Dr. H. Saha and others at the Nilratan Sircar Medical College, Calcutta.

Cardiac output of nine patients with anaemia and one patient with thyrotoxicosis was estimated by direct Fick's method during rest and also during exercise. The exercise was performed on a variable load bicycle ergometer fixed vertically at the foot end of the x-ray table. Each patient took the exercise close to the limit of his tolerence for 5 minutes and the steady—state observations were recorded.

The haemoglobin content of the blood of the anaemic patients varied between 4.7 g. and 11.8 g. per cent. The 'Resting' ventilation and oxygen consumption of these patients were within normal limits. The percentage saturation of the Mixed Venous Blood varied between 49.2 per cent and 60 per cent which is below the average normal value. Arteriovenous oxygen difference was low (1.5 c.c. to 3.5 c.c.per cent) and the 'Resting' Cardiac Index was high (3.2 lit/min/M_2 to 8.8 lit/min/M_2) in all cases except the one with haemoglobin value of 60 per cent.

On exercise, the increase in oxygen uptake varied from 285 c.c./min/M_2 to 736 c.c./min/M_2 during 'steady-state' in different patients depending upon the level of work performed.

Oxygen saturation of the arterial blood decreased slightly in 3 and remained almost constant in the rest. The Mixed Venous Blood saturation, as expected, decreased on exercise. In one patient, who had 27 per cent haemoglobin and performed an exercise demanding oxygen five times his resting value, the saturation of the Mixed Venous Blood fell down to a surprisingly low value of 9 per cent Cardiac Index on exercise varied between 5.6 lit/min/M_2 and 19 lit/min/M_2 depending upon the grade of exercise performed. Compared with the data published by Bishop *et al* (1954) the cardiac output of the patients during exercise was higher than the predicted value in all the anaemic patients.

In this small series of patients no relation could be established between the degree of hyperkinesia and the deficit of haemoglobin in the blood.

Only one patient with thyrotoxicosis (BMR+18 per cent) was avail able for investigation. His cardiac output during rest and on exercise were on the higher side of the normal limits.

219. Enquiry on free amino acids in certain regions of monkeys' brain under Dr. S. I. Singh and Dr. C. L. Malhotra at the Lady Hardinge Medical College, New Delhi.

After standardising the techniques with known amino acids, preliminary studies were carried out on the free amino acid pattern in different parts of the monkeys brain. Qualitatively the distribution of free amino acids is similar in the frontal lobe, amygdala, temporal lobe, hippocampus and cerebellum. Hypothalamus seems to differ from the other areas of the brain as far as its free amino acid pattern is concerned.

The studies so far carried out on two monkeys, reveal that all portions of the brain are characterised by high concentrations of glutamic acid, glutamine, aspartic acid, cystine, glycine, gamma-aminobutyric acid and taurine. Alpha-alanine, serine, threonine, methionine, valine and leucine are present in low concentrations.

220. Enquiry on the "Biogenesis of Alkaloids in Rauwolfia Serpentina plants" under Dr. B. C. Bose at the M. G. M. Medical College, Indore.

In recent years, rauwolfia alkaloids came into prominence for their pharmacological and therapeutic uses. No work, however, was carried out on the biogenesis of alkaloids in this plant. It was, therefore, proposed to study the mechanism of synthesis of alkaloids in this plant by *in vitro* and *in vivo* techniques. The nature of the intermediate compounds and the enzymes involved in the above process were also to be investigated.

To start with *in vitro* studies using a group of amino acids as precursors were taken up. The plants selected for the study were of about 10 months of age. Samples of roots, stems and leaves were incubated in salt buffer solution at 37°C. for 24 and 48 hours using amino acids-tryptophane, tyramine, p-amino benzoic acid, alanine, glutamic acid, histidine, arginine and tyrosine. The content of total alkaloids in control sets and test samples was analysed by the modified method of Horhamner *et al* (1954). The quantitative estimation of reserpine in samples was carried out spectrophotometrically. This method which permits detection of micro quantities (of 0.25/ug./c.c. with an accuracy of ± 4 per cent), has been satisfactorily developed in this laboratory for the above work. It involves the extraction of reserpine with alcohol, removal of interfering alkaloid with hydrochloric acid and finally extracting reserpine with chloroform from sulphuric acid solution.

From the *in vitro* observations, it was found that proline, alanine, aspartic acid, tyrosine, tryptophane, and phenyl alanine produced a significant increase in the alkaloidal content of roots, maximum being 52 per cent increase with tyrosine. Amino acids—histidine, glutamic acid, p-amino benzoic acid and arginine did not produce any appreciable effects. As regards stems and leaves, it was observed that only tryptophane produced a significant increase of 20 per cent and 16 per cent respectively. Of the above three parts investigated, roots showed the maximum of 3.66 mg,/g., as against 2 mg./g., in stems and 0.8 mg./g., in leaves indicating thereby that roots probably carry out the dual functions of synthesis as well as storage of the alkaloids.

As regards the reserpine content after treatment with the above amino acids, it has been observed that proline, tryptophane, alanine and phenyl alanine produced an increase in roots, maximum being 44 per cent with proline. Tyrosine, arginine and p-amino benzoic acid had no effect on the synthesis of reserpine in rauwolfia roots.

By paper chromatographic technique, it was found that roots of *R. serpentina* contained a larger number of alkaloids in greater quantities as compared to the stems and leaves. Presence of reserpine could not be demonstrated in the leaves although it was present in roots and stems. Serpentine was found to be present in roots, leaves and stems.

221. Enquiry on the location and isolation of the pupation 'Hormone' in the Indian house fly Musca, nebulo Fabr, under Dr. P.J. Deoras at the Haffkine Institute Bombay.

The report is a summary of the work done on the morphology and histology of the ring gland, which forms the first part of the above scheme. The object was to locate the glands responsible for the secretion of the pupation hormone in *Musca nebulo* and to study the histological changes in the gland during the secretory phase.

House flies were collected from different localities in Bombay, identified, and the desired species was bred in large numbers in the laboratory. The morphology of the ring gland in larvae was studied in whole mounts stained with borax carmine, and in direct dissections supravitally stained with methylene blue. The histological details were observed in serial sections. The brain together with the ring gland was fixed in Bouin's or Helly's fluid and stained with Heidenhains' iron haematoxylin-eosin, Gomori's chrome alum haematoxylin phloxine or Masson's trichroma.

The ring gland of the mature larva lies dorsal to the brain in a more or less horizontal position with the anterior part extending in front of the brain and the posterior part overlapping the cerebral hemispheres behind. The gland is easily seen in dissections as an elongated ring, bordered on either side by the cerebral tracheae to about half its circum ference at which point these tracheae diverge and join the main lateral trunks towards the anterior end. A short trachea which traverses the substance of the ring along the anterior half, unites these two cerebral tracheae. The anterior end of the gland is broadly triangular in shape and from its apex a slender ligament runs dorsal to the aorta to which it is attached. Posteriorly, the two sides of the ring, unite over the cerebral hemispheres, the recurrent nerve entering the gland at this region. In whole mounts of the ring gland of larvae after cessation of feeding large rounded deeply staining nuclei are prominent- the cell boundaries being indistinct. Such nuclei are particularly consepicuous on the lateral parts of the ring. The gland surrounds the aorta which lies dorsal to it posteriorly and ventral to it anteriorly. The wallof the aorta is intimately associated with the ventral surface of the gland both at the posterior and anterior regions, but is free at the sides.

The nerves to the glands were studied in dissections, supravitally stained by methylene blue. The recurrent nerve arises from a triangular ganglion, runs dorsal to the oesophagus and just behind the cerebral commissure divides into a dorsal and a ventral branch. The latter pages under the brain dorsal to the oesophagus ending in the frontal ganglion. The dorsal branch runs dorsal to the cerebral commissure and enters the posterior aspect of the ring gland from below. From the brain two pairs of nerves emerge to enter the ring gland ventrally at the posterior end of that organ. The inner pair consists of short slender nerves and careful observation under high power reveals that what actually appears as the inner nerve is composed of two fine nerves which are united only at their point of emergence from the brain where they appear to crost each other and then run as separate strands to enter the ventral aspect of the ring gland. The outer of these two can be seen extending along the sides of

the gland to a short distance. Each of these fine nerve strands, has a small swelling at approximately two thirds of its length from the brain with as single prominent nucleus in this region. The outer nerves are thicker and arise from the posterior inner aspect of the cerebral hemispheres just in front of the cerebral trachea which also enter, the brain in this region. These outer pairs of thick nerves enter the ring gland lateral to the inner pair. This outer pair is the nervous corporis cardiaci II; and the inner pair the nervous corporis cardiaci I.

A comparative study of the ring gland in the three larval stages shows that the lateral component of the ring undergo maximum development in the last instar larva, During the prepupation period the cells of the lateral sides of the ring enlarge considerably and show secretory products. This visible change can be correlated with the fact that this part of the ring secretes the hormone responsible for pupation. In larvae about to pupate the gland shows signs of structural disintegration and by the time the adult emerges from the pupa the lateral components of the ring are completely lost.

Ligature Experiments :-Following the classical experiments of Fraenkel(1935) the third stage larvae were ligatured with fine silk thread at different regions in different stages to find out the critical period when the pupation hormone is secreted. Larvae were lightly anaesthetised before the ligature was applied. In each series, larvae of the same age were taken, and controls from the same group were kept to study any possible effects of exposing them to ether.

In the first series, larvae 15 hours prior to pupation were ligatured. In one group the ligature was applied in front of the brain between segments three and four, while in the other group the ligature was applied behind the brain between segments seven and eight. 24 percent of the larvae died at varying periods after the ligature was applied (before pupating) while only five per cent of the controls died without pupating, and another five percent failed to pupate even after 24 hours. In both tne groups both halves pupated. Pupation was considered to have occurred when the characteristic hardening and darkening of the cuticle took place. A number of these ligatured pupae were dissected after 24 hours in the majority of cases, development had continued.

In the next series larvae 24 hours before pupation were taken for the experiments. These were divided into three batches. In larvae of one batch the ligature was applied behind the brain while in another group the ligature was applied in front of the brain. The third batch was kept as controls. In the first two groups again the mortality was high (about 30 per cent) while in the controls mortality was very low (4 per cent). In larvae ligatured behind the brain only the anterior half pupated, approximately 26 hours after the ligature was applied. In a number of such larvae the posterior half lived for more than 48 hours but showed no signs of pupation. In the second group ligatured in front of the brain only the posterior half pupated. The anteaior half becoming dried and shrivelled as early as 12 hours after the ligature was applied. As before a few of the pupated halves were dissected 24 hours after pupation had taken place and in most cases development had continued to

take place. These results fully agree with Frankel's observations on Calliphora. Further work is being done to narrow these limits so that the approximate critical period can be known in the development of the house-fly larvae.

The above observations show that pupation is induced by a hormone secreted at the anterior region of the last instar larva. There is a critical period when the hormone is secreted. Once it has been liberated in the body, pupation can take place even in the absence of the nerve centre and the ring gland. The lateral part of the ring, considered to be homologous to the peritracheal glands of other insects, secretes the pupation hormone. Work is being done to experimentally induce pupation in larvae at varying stages in their development, by the injection of haemolymph from pupating larvae and by the transplantation of the gland.

222. Enquiry on the isolation of active principle and pharmacological studies of Lagerstroemia speciosa (Arjuna) seeds, roots and fruits under Dr. B. B. Gaitonde at the Grant Medical College, Bombay

Summary of the Preliminary Work.

Lagerstroemia Flos Keginae (Arjuna) (Lithraceae).

Arjuna is described as a narcotic (seeds) Astringent (roots) and purgative (barks and leaves). The bark of this plant is studied for pharmacological action. The bark was powdered and various fractions were separated for pharmacological studies. Thus three fractions - water soluble, Petroleum Ether Soluble and Alcohol soluble were prepared and studied for their effects in animals. Watery Extract :—Watery extract did not produce any narcotic activity in albino rats in a dose of 5 mg./kg. body weight. It was also ineffective as anticonvulsant against Leptazole given intraperitoneally in a dose of 80 mg. kg. In a dose of 25,10 and 50 mg. per kg. it produced a graded fall in B. P. of anaesthetised cats, followed by a rise. In a higher dose it produced a fall which was associated with a phase of Apnoea which lasted for about 10 to 15 seconds.

Alcoholic Extract :—0.5g. kg. of this fraction produced a slight sedation and anticonvulsant effect in albino rats.

The extract was found to be farely toxic when given I.V. in anaesthetised cats. Thus in a dose of 5 mg kg it produced initial phase of a marked respiratory slowing followed by a techicardia ; rise of B. P. and a phase of tachypnoea. In a dose of 50 mg. kg. it produced cardic arrest. It was given orally to a group of rats to observe a diuratic response. Thus, in a dose of 1 mg. kg. orally it produced a significant rise in urine output over control rats. during a period of observation extending from five hours to 24 hours.

Petroleum Ether Extract (Oily fraction) :—fraction. The oily fraction was emulsified in Tween 80 and water and used in experimental studies.

When studied for its effects on B. P. of anaesthetised cats it was found to produce a very marked but evanascent fall in B. P. in a dose of 1 mg; 5&10 mg. kg.

Further work on sedative diuretic aud anticonvulsant properties of all these fractions was planned, but the Enquiry was terminated.

223. Enquiry entitled "Electro-retinogram for white and coloured lights in rod and cone retinae of some nocturnal and diurnal animals" under Dr. J.N. Prasad at K G. Medical College, Lucknow.

The object of the study was to investigate:

1. If the rods respond to the coloured lights, and
2. If the cone or rod retinae in different animals respond in an identical way.

The animals were divided into three groups :—

1. Those with mixed retina.
2. Those with cone retina.
3. Those with rod retina.

To start with the animals belonging to group (I), i.e. mixed retina are being studied. Cat and dog belong to this group. The observations are made for different intensities of light of different colours.

The intensities of light tested are 25, 50, 75, and 100 f.c. The lights are white, red, green, blue, yellow, and orange. Six observations are made with each light. The results obtained so far as follow :

DOG :

White light : Good response to all intensities of light. This response appears to remain more or less constant for all intensities.

Green light : Poor but definite response. The response varied for different intensities. In one dog there was no response.

Red light : Poor but definite response like that for green light.

Blue light : Good response, better than that for white light.

Yellow light : Good response, but poorer than that for white light (only 3 dogs used).

Orange light : Good response in the only dog tested.

. CAT:

White : Very good response, better than that shown by the dog. Generally the intensity of response increased with the increase in the intensity of light.

Green : Very good response in the two cats tested.

Red : Good response.

Blue : Very good response, much better than that in dogs.

Yellow : Good response in the only cat tested.

224. Enquiry on qualitative and quantitative studies on spermatogenesis and its variation during oestrogen administration and experimentally induced liver damage under Dr. J. C. Sachdev at the M.G.M. Medical College, Indore.

Normal male healthy albino rats were maintained on a synthetic diet containing 20 per cent protein for a period of 30 days. The animals showed an average gain in body weight of about 20 grams, and the testes were pale pink in colour and soft in consistency. Not much variation in the size of the right and left testes was observed, but left testis was found to be slightly heavier than the right. The average size of the testis was 12x15 m.m. and the average weight of the right testis was 0.918 g. and of left 0.97 g.

Quantitative studies of different stages in spermatogenesis by microscopic examination of 2500 tubular cross sections using Roosen-Rung classification gave values of 3.8 per cent fer stage I, 4.8 per cent for stage II, 14.3 per cent for stage III, 5.3 per cent for stage IV, 8.7 per cent for stage V, 34.7 per cent for stage VI, 12.7 per cent for stage VII and 16.2% for stage VIII. These values correspond more or less with those obtained by Roosen-Rung.

225 Indigenous Drugs enquiry under Col. R. N. Chopra at the Regional Research Laboratory, Jammu.

I. Pharmacological screening of indigenous medicinal plants was continued and the following four plants were investigated:—

1. *Lochnera rosea* Reichb (Rattanjot).

Total alkaloids were isolated from the root bark of the plant and their pharmacological and antibacterial properties studied. The alkaloids showed significant and sustained hypotensive action which was further enhanced in experimental hypertension. The drug also produced relaxation of plain muscle and sedative effect on the central nervous system.

The alkaloids inhibited the growth of *V. Cholrea* Inaba and *M. pyogenes* var. *aureus* but showed no inhibitory action against enteric group of organisms. Further studies are in progress.

2. *Inula royleana.*

In continuation of our investigations on *Inula royleana*, besides Roylene, two more alkaloids and a yellow coloured neutral substance, have been isolated. The alkaloid which is present in larger proportion, is amorphous in nature. The pharmacological action of these alkaloids was studied and it was found, that the amorphous alkaloids showed a well marked depressant effect on respiratory system. Blood pressure was depressed and smooth muscles relaxed. The alkaloid also showed a marked curariform activity.

3. *Ammi visnaga.*

It is a perennial plant, which grows in waste lands of the eastern Mediterranean and particularly on the Nile Delta. The plant has been successfully introduced in Jammu. Pure alkaloid khellin was isolated from the seeds and its pharmacological activity compared favourably with Viscardan Khellin preparation of B.D.H.

4. *Chenopodium ambrosioides* var *Anthelminticum.*

It is an exotic plant from North America which has been successfully cultivated in Jammu. The plant yields an oil 1 per cent by weight containing 66-70 per cent ascaridole, which has been tested for its anthelmintic activity.

II. Screening of antibacterial properties of Indigenous drugs :—

The following plants were screened for the antimicrobial activity :

1. *Artabotrys suaveolens.*
2. *Carum carvii*
3. *Eugenia jambolana.*

4. *Foeniculum vulgare.*
5. *Jatropha curcas*
6. *Acacia catechu*
7. *Acacia arabica*
8. *Juglans regia.*

The alcoholic extract of *A. catechu* is inhibitory to *B. typhosum* in 400 ug./ml. and *M. tuberculosis* in 100 ug./ml. The alcoholic extract of hernel of *J. regia* inhibits *Sh. dysenteriae* Shigae and *M. tuberculosis* in 250 ug./ml. and *B. typhosum* in 100 ug./ml.

Detailed investigation on *Peristrophe bicalyculat* showed that essential oil obtained by steam distillation possesses a specific *in vitro* activity against *M. tuberculosis* without effecting the growth of other micro-organisms.

226. "Investigation into the study of adrenal cortical harmones" under Dr. B. Mukerji at the Central Drug Research Institute, Lucknow.

Work done during the year under report can be summarized under the following heads :—

1. *Studies on the nature of LH action on the adrenal cortex.*

Triiodothyronine (TIT) caused a stimulation of the adrenal cortex of castrated rats presumably through a stimulation of pituitary ACTH output. The animals were first exposed to a high titre of endogenous LH by allowing sufficient time to elapse between castration and TIT treatment. Administration of testosterone proplonate (TP) along with TIT significantly reduced the adrenocortical response evoked by endogenous ACTH. This suggested that the primary influence of LH was to sensitize the adrenal cortex to ACTH action ; TP by inhibiting pituitary LH release eliminated this sensitizing effect. Further, any possible direct influence of TP on pituitary ACTH activity was eliminated by the fact that when given alone it (TP) failed to influence the adrenal cortex of castrated rats.

2. *Studies on the action of adrenal cortical hormones on functioning of the testis.*

Aldosterone arrested the development of testis in young rats as shown by low testis weight and retardation of spermatogenesis at the primary spermotocyte stage. The differentiation of Leydig cells was also inhibited but atrophic changes were absent in the interstitial elements. Total cholesterol content of the testis showed no alteration on absolute basis but the relative concentration was elevated. The seminal vesicles did not show any appreciable change in weight or histology. DOCA also caused some retardation of testicular development in similar rats as indicated by low testis weight and arrest of spermatogenesis at the spermatid stage. The differentiation of Leydig cells was also somewhat inhibited but no atrophic changes were seen in the interstitium. The effects on testicular cholesterol and the seminal vesicles were comparable to those of aldosterone. It was concluded that the nature of response of the testis to the two corticoids was the same but aldosterone was more active than DOCA. Further, on the basis of per cent lowering of absolute testis weight it was estimated that in the dosage used aldosterone was about twice as active as DOCA. On the other hand, it was known that aldosterone possessed about 30 times more activity on electrolyte metabolism as compared to DOCA.

227. Studies on twins and consanguinity under Dr. L. D. Sanghvi at the Indian Cancer Research Centre, Bombay.

Study on twins :

1. In order to obtain an accurate incidence of twinning at birth and the proportion of zygosity, records of three large maternity hospitals in Bombay City were examined covering a six-year period (1952-57). The survey included 104,338 births.

2. Thirteen more pairs of twins were added to the series bringing the total examined so far to 140. These records were examined critically and a revised list of characters was prepared for a study of a fresh series of twin pairs to be taken up for the refinement of diagnostic criteria for zygosity.

3. Theoretical methods are being tried to determine the probability of monozygosity or otherwise and to evolve a linear function to classify doubtful cases.

Study on consanguinity :

1. Seven thousand two hundred forty-nine marriages in the rural areas of Andhra Pradesh, were studied for the rates of consanguinity. Exceptionally high rates were encountered in this area.

2. A study has been undertaken, in association with Dr. Gopal Rao at the Osmania Medical College, Hyderabad, to relate the rates of consanguinous marriages to the rates of still-birth and congenital malformations.

3. A study of 21 cases of leukoderma in 16 Parsi families revealed 5 families with first cousin marriages. This rate was significantly higher than the rate in the community indicating the presence of recessive genes as one of the aetiological factors.

228. Enquiry on hypothalamic and endocrinal control of physiology of reproduction under Dr. B. K. Anand at the All-India Institute of Medical Sciences, New Delhi.

The first step in this enquiry was to separate the degradation products of oestrogen and progesterone in monkey's urine and to measure them quantitatively, so as to establish the normal pattern of their excretion during the different phases of menstrual cycle. Techniques were standardized for extracting, separating and purifying from she monkey's urine all the degradation prodocts of both oestrogen and progestrone separately which were designated as "E" and "P" fractions, respectively. They were then quantitatively measuaed by spectrophotometer by studying the absorption curves in the ultra violet light. After finalization of these techniques, studies were undertaken with 5 monkeys to graphically represent the secretory activity of their ovaries by estimating "E" and "P" fractions appearing in their urines during normal menstrual cycles. After these normal cyclic excretory curves are worked out for 3-4 cycles, stimulation and ablation experiments will be undertaken in these monkeys to study the relationship of the hypothalamic region to this cyclic secretory activity.

Attempts were also made to further fractionate the "E" and "P" fractions and to identify the compounds so separated spectro-photometrically. It has, so far, been possible to identify oestrone, oestradiol, and oestriol in the "E" fraction : and progestrone and pregnanediol in the "P" fraction.

229. Study on spermicidal drugs and oral contraceptives under Dr. M. L. Gujral at the K. G. Medical College, Lucknow.

Sixteen fractions of *Mallotus phillippinensis* including rottlerin, isorottlerin and acetyl-rottlerin were prepared and tested for their possible antifertility effect on rats. The drug was given to female rats for a period of 8 days. All animals used in the experiment were known to be fertile in a previous observation. Vaginal smears were taken daily and examined to mark the stage of oestrus cycle, mating and nature of pregnancy. Female rats were put for mating after the period of drug administration was over and were observed for effect of the drug on their fertility.

The results of these tests show that rottlerin and its acetyl derivative are active while isorottlerin (ring closure of the chalkone to flavone) seems to be either totally inactive or only very slightly active. Even though rottlerin is insoluble or only very slightly soluble in cold solvents like petroleum ether, alcohol and ether, it is found that the latter can extract a small amount of it from the 'Kamala' powder. These fractions therefore show a positive test in the biological screening. No steroid has so far been isolated from *Mallotus phillippinensis*.

High doses of the various fractions were employed in this preliminary screening in order to be sure that no fraction was discarded as inactive because of an inadequate dose. Study with smaller doses is now being carried out.

230. Field trials with foam tablets under Dr. V. R. Khanolkar at the Contraceptive Testing Unit, Indian Cancer Research Centre, Bombay.

I. *Preliminary preparation :*

(a) In Naigaum area, a population of about 3,000 was surveyed and a house-to-house personal interview carried out by the social workers. Schedules were filled in the case of 686 couples. These were all potentially fertile couples where the women were between the ages of 15 and 40 years and had at least one child not more than 3 years previously.

(b) During the survey, 16 lectures and film shows were held in Naigaum area. At some of these meetings, guest speakers were invited to address the gathering. A meeting of the doctors practising in Naigaum was also held in order to acquaint these practitioners with the work being done at the clinic.

II. *Distribution of birth control appliances :*

During the survey of this area, 235 women actually attended the clinic for advice. Of these, 72 were given diaphragm and jelly and 132 were given foam tablets. In each of the women, who came for birth control advice, a gynaecological examination was carried out along with certain special investigations, such as exfoliative cytology, colposcopy and Schiller test.

III. *Follow-up of those using contraceptives.*

A follow-up study of all the couples using foam tablets or diaphragm and jelly is carried out and information is obtained regarding the use of the method, difficulties encountered, if any, regularity of menstrual periods and occurrence of pregnancies.

Proposed plan of work for 1959-60.

The distribution of appliances will be continued in the area surveyed and a follow-up of these women will be carried out. Field studies with the foam tablets produced at the Indian Cancer Research Centre will be continued along with studies using other foam tablets and jellies recommended by the Director General of Health Services, for comparison

231. Field trials with foam tablets under Dr. D. Anand at the Health Unit, Orientation Training Centre, Najafgarh.

The Indian Council of Medical Research has approved this scheme only in November 1958. The staff are being recruited.

The trials will be carried out in the rural areas around Najafgarh. Two groups of villages, one with a population of about 10,000 for the trial of foam tablets and the other of 8,000 population to serve as a control will be selected. The programme will consist of :—

(1) Offering contraceptive services as part of the health services of a primary health centre.

(2) Promoting the use of contraceptive by a health education programme.

(3) Using reasonably reliable methods for assessing changes in attitude, acceptance and use of contraceptives, and measuring changes in birth rates.

The work will be carried out in accordance with the aims given in the Indian Council of Medical Research document "Field Studies on Contraceptives" dated August 27, 1958 in three phases, (1) Preliminary preparation, (2) Application of methods and (3) Evaluation of results.

232. Field trials with foam tablets under Dr. (Kumari) A. D. Engineer at the Medical College, Lucknow.

This study is one of the four being carried out on a large scale to evaluate the effectiveness, acceptibility and harmlessness of contraceptive methods in reducing the birth rate, of rural population.

The idea initialey was to try various contraceptive methods, but as the study is to be restricted to a relatively illiterate and economically poor group, all the mechanical methods of contraception are deemed unsuitable, and the programme is, therefore, confined only to the use of intravaginal foam tablets.

Two groups of villages were selected for the study. One group will be used for the experiment while the other will serve as the control. The experimental villages have a population of approximately 10,000, and are all within 12—14 miles from Lucknow City. The control group of villages is similar in every respect to the experimental group, and has approximately the same population, but they are selected from a different direction to ensure that no knowledge of contraceptives reaches them from the experimental group.

The study at this centre commenced in September, 1958. The experimental villages were divided into 4 groups and two social workers, one male and one female, were recruited for each group.

The study is being carried out in 3 stages :—

(i) Preliminary stage of preparation.
(ii) Application stage, which consists of distribution of contraceptives to eligible couples and their follow-up.
(iii) Evaluation of results.

The preliminary stage consisting of listing of households in the experimental and control areas, and determination of the prevalent birth and death rates in both groups at the commencement of the study, has already been completed.

The application stage of the study has just been commenced, and suitable couples are being screened by the social workers and brought to the research worker attached to the survey for the distribution of contraceptives. Those couples who accept the foam tablets are entered as active cases and are being visited every fortnight by the social workers.

VIRUS DISEASES

233. Polio Research Unit under Dr. P.V. Gharpure, at the Grant Medical College, Bombay.

Main emphasis of the investigation has been on (i) the various aspects of epidemiology of poliomyelitis and (ii) the pathogenicity of polioviruses.

A. *Epidemiological Studies*:

1. *Bombay :*

The general pattern of poliomyelitis in Bombay has remained unchanged with regard to :—

(i) Geographical distribution, (ii) Age distribution, (iii) Sex distribution, (iv) Seasonal incidence and (v) Mortality.

Two hundred fifty two clinical cases of poliomyelitis occurred during the period 1-9-1957 to 31-8-1958. Of these cases, 146 were males and 106 females. Thirteen were declared as non-polio after due investigation. Only 4 cases were recorded in individuals above the age of 14 years.

Non-Indian Cases : One in a child aged 9 years. Nil in adults. Thirty-eight were imported cases including one non-Indian.

2. *Dohad* :

Studies were commenced in March 1957 and were concluded in March 1958. Six visits were paid to Dohad, and 185 sera and 44 stool samples were collected and tested. The results of virus isolation and serological tests corroborate the findings reported in last year's annual report.

3. *Darjeeling* :

(i) Six stool samples and 21 sera were received and tested.

(ii) The findings indicated a low endemicity of poliomyelitis in the area.

(iii) The age of occurrence of cases was distinctly higher in this area than that noted elsewhere.

(iv) Serological findings showed that a fair proportion of the population is susceptible to one or more types of the poliovirus.

B. *Virus Studies*:

Seven hundred forty-eight samples of faeces were collected for virus studies of which 649 were tested in tissue culture. Two hundred twelve cytopathogenic agents were isolated, of which 77 were polioviruses and 18 belonged to the nonpolio group. Of the 77 polioviruses, 43 were type 1, 19 were type 2 and 15 were type 3. One hundred seventeen are yet to be identified.

Pathogenicity of poliovirus : Work was planned for the detection of avirulent strains. Out of 56 isolations in tissue culture from stools of apparently healthy children, 16 were proved serologically to be polioviruses. Of these 10 were type 1, 3 type 2 and 3 type 3. Suspecting that this group contains isolations with a mixture of viruses, the following investigations were made.

Ten pools were prepared with 4 to 5 members in each pool and inoculated intracerebrally using a single animal for each pool. five pools turned out to be paralysis producing. The study of individual members of these groups is still to be undertaken.

The group of 16 serologically proved polioviruses, referred to above, was next taken up. Of those, so far 13 were inoculated in monkeys, out of which 3 strains were found to produce no paralyses—one type 1, two type 3. The virus was recovered from the spinal cord of one animal. The positive cord material has now been inoculated in 3 animals. The original 3 strains have also been put in a series of 3 animals, for each strain. The experiment is in progress.

The tissue culture fluid of these 3 strains has been freeze-dried and viability in the dried state is being studied.

As a corollary to the study of the excretion of the virus, preliminary study on the virus content in rectal swabs of monkeys coming to the animal house was undertaken. So far, 211 rectae swabs were collected, of which 112 were tested in tissue culture and 65 cytopathogenic agents isolated.

Antisera against these simian strains and other cytopathogenic agents are being prepared.

Study of non-polio viruses isolated:

Seventy-seven cytopathogenic agents were serologically proved to be non-polio, of which 37 were from clinical cases of polio and their contacts and 40 from apparently healthy children. 21 strains were studied so far and only 11strains could be identified serologically. One of these strains was coxsackie B1, three B5 and seven B7.

Samples of stools were also collected from 46 infants and 30 young children.

A limited amount of investigation on the existence of avirulent strains has been undertaken. For this, stools of very young children from localities where avirulent strains were isolated, are being collected So far 30 samples have been collected. All have been tested and 13 showed presence of cytopathogenic agents.

Preliminary work on the problem of vaccine product[illegible] as been undertaken at the Haffkine Institute.

234. Respiratory and intestinal viruses-unit under Dr. N. Veeraraghavan at the Pasteur Institute, Coonoor.

I. Tissue Culture Methods.

During the year the techniques of tissue culture for growing freshly isolated monkey kidney and human amnion cells and propagation of standard cell lines were standardized. Monkey kidney and human amnion cells are now regularly prepared. In addition, Hela, KB and a strain of intestinal epithelial cells are being maintained.

II. Respiratory Viruses.

A. ADENOVIRUSES

1. An investigation was undertaken to study the occurrence of the respiratory viruses of the adeno-group during the recent epidemic of influenza. A large number of specimens were collected during the main epidemic in 1957 and a smaller number from localized outbreaks caused by the same virus in 1958.

All the 84 specimens were passed thrice through amnion cells, 37 in Hela cells, 29 repeatedly in KB cells and 44 in monkey kidney cells. Out of the 84 specimens examined. adenovirus was isolated from only one case. Neutralization experiments using monospecific antisera indicate that the virus belongs to type 7.

2. Monospecific antisera against adenovirus types 4 and 7 were prepared during the year.

B. MEASLES VIRUS

Three strains of measles virus have been isolated in human amnion cell cultures. Cytological studies are being carried out on cell sheets grown on cover slips and infected by these strains.

III. Intestinal Viruses.

A. ENCEPHALITIS

The main research programme during the year has been a study of the aetiology of cases of encephalitis which occurred in Delhi in 1957, and in an epidemic form in Nagpur, in June and July 1958. Over 460 specimens have been collected and are being studied. A brief summary of the results is given below :

DELHI CASES

Five viruses were isolated from the specimens tested so far, from cases of encephalitis in Delhi, all of them from faeces. Three of them

were identified as polio virus, type 1. The other two were labelled as ECHO viruses as they were not neutralized by any of the three standard polio antisera and were not pathogenic to less than 24 hour-sold suckling mice. These viruses were aetiologically related to the illness as the convalescent serum samples neutralized the virus to a titre of log. 1.5 to 1.2, whereas the sera from acute stage of illness did not show the presence of neutralizing antibodies in a dilution of 1 in 10 and above.

Two specimens of faeces and 7 specimens of cerebrospinal fluid from 9 fatal cases yielded no virus in monkey kidney cells.

No virus could be isolated from any of the 32 samples of cerebrospinal fluid tested in tissue culture.

Thirteen faecal samples were also inoculated into suckling mice with negative results.

NAGPUR CASES

Seven viruses were isolated from 5 specimens of faeces and 2 specimens of throat swabs. Two more throat swabs appeared to be positive for virus isolation. These are to be confirmed. Four of the viruses were not neutralized by any of the three standard polio antisera. The others are yet to be tested against these sera. Studies in connection with the identification of the viruses are in progress.

B. POLIOMYELITIS

Using infected tissue culture fluids containing high titre of the virus immune sera have been prepared in monkeys against the three types.

C. MISCELLANEOUS

1. The following laboratory procedures have been standardized :

   (i) Metabolic inhibition test for the assesment of polio antibody in sera.

   (ii) Plaque formation of polio viruses in monolayer monkey kidney cell cultures.

   (iii) A micro method for viral complement fixation tests using antigens prepared from infected tissue culture fluids.

2. Standard strains of polio, Coxsackie and some of the ECHO group of viruses are being maintained in tissue culture.

3. Preparation of immune sera against standard Coxsackie and ECHO virus strains is in progress.

235. Rabies enquiry under Dr. A. K. Thomas at the Central Research Institute, Kasauli.

I. In an experiment to determine the optimal dosage of anti-rabic serum, groups of animals were exposed to salivary gland street virus and varying dilutions of serum (neutralising index 1:4000 × 500) administered 24 hours after the exposure, followed by a fixed dose of vaccine for 14 days from next day onwards. It was found that protection could be obtained in a fair range of dilutions of the serum.

II. To determine the relationship between the dosage of serum and vaccine, a fixed dose of serum followed by varying doses of vaccine ranging from 1.5 ml to 0.25 ml was administered for 14 days to different groups of animals exposed to street virus. It was found that in this case also a wide range of dosages of vaccine was effective in protecting the animals.

III. Experiments on the same lines as above were carried out to correlate the circulating antibody level with the behaviour of infected animals by bleeding them at different periods after exposure and during the period of treatment. The neutralisation tests of the different samples of serum are not yet completed.

IV. The effectiveness of serum treatment at different periods after exposure to street virus followed by vaccine treatment for 14 days is being tried in guinea-pigs.

V. Circulating antibody levels in serum of persons receiving different courses of treatment were estimated. A course of 2 cc for 7 days does not appear to produce any detectable antibody level at 1:16 and 1:32 dilutions of serum against a virus dosage of approximately 30 LD_{50}. A booster dose of 2 cc, 14 days after the first dose, however, produces a detectable antibody level.

The course of 5 cc for 14 days showed, except in 2 cases out of 10, protection in the dilutions tested.

In 2 cases where serum was administered followed by 10 cc of vaccine for 14 days, the antibody levels were found satisfactory.

VI. Attempts to remove the paralysis producing factor from the anti-rabic vaccine by treatment with Bentonite and Calcium Phosphate Gel. were not successful.

VII. Immunological tolerance to brain tissue as a method for prevention of experimental encephalomyelitis has been tried with partial success in guinea-pigs. These studies are being continued.

236. Rabies enquiry under Dr. N. Veeraraghavan at the Pasteur Institute, Coonoor.

It has been reported earlier that the optimum dosage of serum and vaccine to be administered for good results seems to vary with the quality of the serum and the antigenicity of the vaccine.

In view of the above findings it seemed essential that before deciding on the optimum dosage of serum, it is necessary to ensure uniformity in the antigenicity of the vaccine. It is well known that batches of vaccine prepared under identical conditions vary in their virus content as well as antigenicity. Work has been in progress continuously at the Pasteur Institute, Coonoor, to evolve a method by which a vaccine of uniform potency could be regularly produced. As a result of these studies it was found that a vaccine of reasonably uniform potency could be obtained when 12 batches of vaccine were pooled before ampouling. In the pools so far tested the antigenicity was always greater than the reference vaccine. Using pooled vaccine in doses corresponding to human treatment schedule, it was possible for the first time to protect 80 to 90% of guinea-pigs against milder challenge with virulent strains of street virus even when the treatment was started 1 hour after infection. A statistically significant protection could also be obtained against severe challenges of 100 LD_{50}. These findings clearly indicate the superiority of the pooled vaccine. Pooling thus offers a simple method by which antirabies vaccine producing centres could improve the quality of the vaccine they produce. A method has been evolved for pooling batches of vaccine in routine practice and this is being used at the Institute since March 1958.

Using pooled vaccine for treatment and different strains of street virus for challenge, large scale experiment were undertaken to determine the optimum conditions which would give the best results with combined serum and vaccine therapy. These studies indicate :

(i) Antirabies serum alone, while definitely prolonging the incubation period, had no saving effect with the challenge doses employed.

(ii) There is no advantage in giving serum when the challenge is mild as vaccine alone gives good protection in such cases.

(iii) Antirabies serum (PIC) given in 1/25th the recommended dose along with vaccine confers marked protection against severe challenges.

This finding would make antirabies serum therapy a practical proposition because (a) the quantity of serum would be reduced to a fraction (0.01 ml. compared with the recommended dose of 0.25 ml. per pound of body-weight) and (b) the cost of serum treatment would be considerably reduced. This is very important particularly as serum is very expensive.

(iv) According to the present classification any person weighing seventy pounds or more, who is severely bitten, receives 10 ml. of vaccine daily for 14 days, i.e, a total of 140 ml. This works out to 14 doses of 0.15 ml. per pound of body-weight. Experiments were undertaken during the year to determine whether this dosage of vaccine could be reduced when cobined with serum. An interesting finding was that with serum (PIC) given in a dosage of 0.1 ml. per pound of body-weight and vaccine in half the dosage (14X0.75ml.) equally good results could be obtained against severe challenges. This finding is important particulary to countries where the incidence of neuroparalytic accidents following vaccine therapy is high. Experiments are in progress to determine the extent to which the dosage of vaccine could be reduced keeping the serum dosage constant.

Based on the above data combined treatment with serum and vaccine is given at the Institute to patients severely exposed to the risk of infection. The dosage of serum (PIC) given is 0.01 ml. per pound of body-weight. Among the 22 patients so far treated there has been no death.

Extensive studies on the subject carried out at the Institute, clearly indicate that there is interference between the serum and vaccine *in vivo.* The administration of a large dose of serum is, therefore, fraught with danger. Further, the optimal dose of each brand of serum varies with the antigenicity of vaccine administered. It is, therefore, essential that each treatment centre should produce a vaccine of uniform potency and then work out the optimal dosage of its serum in experimental animals before embarking on combined therapy in human beings. Otherwise, it may be doing more harm than good.

237. Enquiry on the adaptation of the Rabies street Virus Strains collected locally to chick embryo at the Haffkine Institute, Bombay.

The work carried out under this enquiry since 1st April, 1958, indicated that it is possible to adapt certain rabies street virus strains to embryonated eggs, when introduced in the yolk sac, as advocated by Koprowski.

Koprowski and Komarew, however, stated that rabies street virus strains can be adapted to embryonated eggs when introduced through Yolk Sac route after a number of intracerebral passages in white mice. In the course of the present investigation, it is observed that not all the strains of the virus could be so adapted to embryonated eggs even after 12 serial intracerebral passages in mice. The low titre of the virus at time of egg inoculation is not the factor responsible for such non-adaptation.

Furter work is in progress.

238. Studies on the incidence, nature and types of Coxsackie virus infections in Bombay City under Dr. N. M. Purandare at the Seth G. S. Medical College, Bombay.

1. *Collection of material and isolation of strains of Coxsackie Viruses.*

One of the seven faecal samples from paralytic poliomyelitis cases revealed the presence of a strain of Coxsackie viruses on inoculation into newborn mice. This makes a total of 40 strains of Coxsackie viruses isolated so far. The strain is being studied for its antigenic pattern by cross neutralization tests in newborn mice.

2. *Isolation of adult-mouse-adaptable virus :*

Attempts to isolate adult-mouse-adaptable viruses from 10 brain samples obtained at necropsies of suspected encephalitis cases at the K. E. M. Hospital, Bombay-12, yielded one positive isolation on intracerebral inoculation of adult mice (4 weeks old). The strain is being studied further.

3. *Effect of aqueous iodine solution on Coxsackie Viruses :*

Four of the locally isolated strains belonging to Dallodorf's group A types 1, 4 and 10 and one unclassified strain respectively were studied for their interaction with iodine solution in two dilutions, 1 : 1,000 and 1 : 10,000. The strains, in the initial dilutions of 1 : 10,000 (100 LD 50 dose) were mixed and incubated with equal amount of Lugol's iodine solution in initial dilutions of 1 : 1,000 and 1 : 10,000, respectively for 5, 10 and 15 minutes at room temperature. The mixtures were inoculated separately into litters of newborn mice at the end of each incubation period. From the observations made so far it appears that iodine in 1 : 1,000 dilution has some deleterious effect on the infectivity of the strains to newborn mice. Only one strain (A-1) was sensitive to iodine in 1 : 10,000 dilution.

4. ***Tissue culture studies :***

(*a*) *Human lipogenic sarcoma tissue culture* :—All the three types of poliomyelitis viruses showed definite cytopathogenic changes in this cell line up to 12 passages studied in the present series. Typical rounding of the cells was noticed within 24 to 48 hours after infection. Two more strains belonging to Coxsackie B-5 and ECHO group of viruses, respectively showed cytopathogenic effects up to 8 passages studied so far. Five cytopathogenic agents were recovered from a total of 42 faecal samples tested for the isolation of polio viruses in the cell line. These agents are being studied further.

None of the strains isolated locally during the studies and belonging to Dalldorf's group A showed any effect on the cells. The 4th passage tissue culture fluids of these strains on inoculation into newborn mice showed the absence of multiplication of these viruses.

(5) *Mouse fibrosarcoma tissue culture.*—None of the viruses belonging to polio, influenza, Coxsackie (strains A-1, 3,4.9,10,12,14, four unclassified strains and B-5), ECHO (types 1,7 and 4 unclassified strains) showed any cytopathogenic effects on the cell line.

Fourth passage tissue culture fluids of Coxsackie strains on inoculation into newborn mice failed to produce paralysis, indicating thereby the absence of virus in the fluids.

5. *Cytological studies of mouse fibrosarcoma cells injected with Coxsackie viruses :*

Mouse fibrosarcoma cells infected with strains belonging to type A-1, A-4, A-10,12, 14 and three untypable strains 126, 165 and 233, were fixed in Bouin's fluid and stained with haematoxylin eosin. Leighton's tube slip method using special flattened tubes, was used for obtaining cells for cytological studies. None of these strains appeared to produce any inclusions either cytoplasmic or nuclear in the cells up to 4, 5 or 6th day after infection. Vacuolation and thinning of cytoplasmic processes were the only signs observed.

6. *Further studies with four untypable strains of Coxsackie recovered during the present studies :*

None of these four strains showed any pathogenicity for adult mice of 21 to 30 days of age. All the strains were found to be antigenically different from each other and were pathogenic for suckling mice of 5 and 8 days of age. Histopathologically they appeared to resemble Dalldorf's group A. On tissue cultures of monkey kidney, human lipogenic sarcoma and mouse fibrosarcoma they failed to produce cytopathogenic effects.

239. Trachoma Pilot Project under Dr. Mohan Lal at the Gandhi Eye Hospital Aligarh.

In October 1956, under the joint auspices of the Government of India and the W. H.O. a trachoma control pilot project was initiated and stationed at the Muslim University Institute of Ophthalmology, Aligarh.

A preliminary epidemiological survey carried out during 1956-57 in 29 villages of Aligarh District, revealed the pattern of incidence of trachoma and the various underlying factors responsible for the transmission of infection in the rural population.

Pilot Treatment (1957-58)

(a) 625 children (with active trachoma) in rural primary schools were given continuous treatment with local application 1 per cent aureomycin ointment applied twice daily by the project staff for 60 working days. Re-examination was done soon after cessation of treatment and again after a follow-up period of 6 months.

Two-thirds of treated cases were cured. In the majority of trachoma cases of stages I and II, the cure when obtained, was during the course of treatment itself. In stage III cases, however, the cure was in the follow-up period. The response in 10-14 year age group was better than that in 5-9 year age group.

(b) 2,443 active cases of trachoma, in 20 villages were given intermittent treatment with 1 per cent aureomycin ointment (two applications a day for three successive days repeated every fortnight for seven times). They were examined soon after cessation of treatment and once again after a follow-up period of 6-8 months. A majority of the cases treated was between 0-9 years, the proportion of Tr. I, Tr. II, Tr. III, being 1 : 1 : 3. In Tr. I cases, 1/3 were cured mostly while under treatment, 18 per cent of Tr. II and 60 per cent of Tr. III were cured mainly during the follow-up period. The response to treatment was better in the older age-group. In Tr. I and II, after initial improvement during the course of treatment, replapses occurred in the follow-up period while the improvement in Tr. III remained stationary.

Follow-up of the cases treated during 1957, *and treatment of those with still active .rachoma.*

(a) 1,120 cases showing still active signs of trachoma were treated again with application of aureomycin 1 per cent ointment twice daily for five successive days in a month for 5 months (March-May and August-September). Half of them were treated by the project staff and the remaining half by the viilagers themselves.

(b) 550 primary school children of the last year's treated group with signs of active trachoma were treated once daily with application of aureomycin 1 per cent ointment, for 60 working days by the school teachers under the supervision of the project staff.

(c) Study of the effect of intermittent treatment by antibiotics on seasonal peaks of bacterial conjunctivitis.

In 5 villages having a total population of 3,054, clinical and conjunctival smear examinations were done on almost all the available individuals. In 3 of these villages, all children under 10 years and those adults suffering from sub-acute or acute conjunctivitis were given one application of 1 per cent aureomycin ointment daily for 5 successive days in a month for 5 months (March-May and August-September) by the project staff. The remaining two villages were kept as control. final examination, clinical and bacteriological, will be done in all the 5 villages in October 1958 and the effect of treatment in controlling the conjunctivitis and indirectly, trachoma will be assessed.

Extended activities of the project at Sitapur (Eastern U. P.).

In one group of 20 villages, 1,403 cases with active trachoma were given treatment with application of achromycin 1 per cent in oil twice daily for 5 successive days every month for 5 months (March-May and August-September). In the second group of 20 villages, 1,001 active cases were kept under control. The final examination in all the 40 villages will be completed by October 1958 and the effect of achromycin 1 per cent in oil, on the course of trachoma and associated bacterial conjunctivitis will be assessed.

240. Study on chemotherapy of virus infections under Dr. V.N. Krishnamurthy at the Vaccine Institute, Bangalore.

Several compounds belonging to the following groups (1) benzimidazoles, (2) hydrazides and aldazones, (3) alkyl and aryl aminothiomethane sulphonates, (4) thiosemicarbazides and (5)-β-(d) galactosides were tested against PR_8-influenza and vaccinia viruses on embryonated eggs. Mice were used for testing the *in vivo* effect of the substances on PR8 influenza virus. 2-Aminothiotriazole, cyanacetylbenzyl thiosemicarbazide and cyanacetyl hydrazide have shown distinct inhibitory activity against vaccinia virus infection in eggs while B.I.T. (Potassium benzyl aminothiomethane sulphonate) has been by far the most active, against PR_8 strain of influenza virus in eggs both *in vivo* and *in vitro*. The latter substance appears to have action on the influenza virus during the early part of the eclipse phase of the viral multiplication. A high degree of inhibition was observed even when the substance was administered 24 hours before viral infection.

241. Study on chemotherapy of Virus infections under Dr. P. L. Narasimha Rao at the Indian Institute of Science, Bangalore.

The screening programme undertaken, was based on the random observations of previous workers and accordingly several benzimidazole derivatives, hydrazides, aldazones, alkyl and aryl aminothiomethane sulphonates, 2-aminothiotriazole, and copper chelating substances, viz., guttiferin and resacetophenone-oxime, were prepared and tested against PR_8 influenza and vaccinia viruses in egg and mouse infections. The results in general support the contention that "benzimidazole derivatives do not at present offer any promise to be of clinical value" and that isatin-thiosemicarbazone, "the only really effective antiviral agent in existence" seems too specific to be of any general use. (cf. The editorial, *Brit. Med. J.*, 1958, 1290). By far the most significant of our findings till date is that concerning potassium benzylaminothiomethane sulphonate, which seems to inactivate both the viruses or their proteins in a way as yet not clearly understood. Clarification of this mechanism may assist in a better choice of chemotherapeutic agents for trials against viral infections.

242. Studies on mutation and recombination of Indian strains of influenza virus under Dr. I. G. K. Menon at the Pasteur Institute, Coonoor.

Ninety two strains of influenza virus Type A, subtypes A1 and A2 isolated at the Govt. of India Influenza Centre, Coonoor between 5th August 1950 and 8th Aug. '58 and several well known foreign and Indian ones formed the material from which strains had to be selected for further study. Based on distinctive differences in their serological behaviour, sensitivity to inhibitors present in normal rabbit and rooster sera and haemagglutination pattern with cells from different species, the following strains isolated at Coonoor, were taken up for experimental work :

A1/C. 1/50	A2/C. 41/57
A1/C. 9/52	A2/C. 48/57
A1/C. 24/55	A2/C. 49/57
A1/C. 34/56	A2/C. 59/57
	A2/C. 93/57
	A2/C. 95/58

In addition to the above local strains, certain well known foreign ones, e.g., PR8, Liverpool mouse-adapted and A/Japan/305 after serial passage in eggs, ferrets and mice and an Asian strain isolated (by Dr. V. N. Krishnamurthy) from the cerebrospinal fluid of a young girl with a previous history of petitmal who developed influenza during 1957 pandemic were also included.

The strains were examined for many other characteristics e.g., effect of chemical reagents like formaldehyde, ether and methyl and methyl alcohol on their haemagglutination titres and various iodine preparations on their infectivity. Differences were observed between strains but the results were not always consistent. It was noticed that formaldehyde markedly reduced the HA titres of purified preparations of some strains like Kolar VI that were also sensitive to inhibitors : but the effect was modified considerably if the unpurified allantoic fluids were used as the source of virus ; some of the Asian strains showed after ether treatment, a remarkable increase in their HA titres against fowl cells while some among them, and almost all pre-1957 strains tested showed a fall in titre. However, these features changed on further egg passage of the strains. The lethal effect of iodine was uniform for all strains.

Some other characters investigated, proved more useful in differentiating easily between strains. A considerable amount of work had been carried out at the Coonoor Centre earlier on the HA spectra of different virus strains against red blood cells from a variety of animals and birds viz., fowls sensitive and insensitive for lipoid haemagglutination, pigeons, turkeyrabbit, guinea-pigs, sheep, monkey, dog, pig, white rat, buffalo, bullock and man. Cells from sheep, pig, buffalo and bullock were not haemagglutinated by any viruses tested. The 1957 Asian strains, however, behave very differently. Tumova and Sovinova from Czechoslovakia reported that the influenzea virus Type A isolated from horses during the 1956 epizootic and the Asian strains isolated from man in 1957 haemagglutinated the red blood cells of horses, sheep, swine

and calf. This observation has been confirmed at Coonoor with the Asian strains up to the passage levels obtained. The pre-1957 strains have been retested at various passage levels against horse cells but with consistently negative results.

An unusual variant isolated in 1952 was studied at various passage levels and was found to breed true, retaining its distinctive serology, difficulty of growth in chick embryo and low haemagglutinin titres even after 13 passages in eggs. Its similarity in the last two points to the Asian strains led to detailed comparisons but no serological relationship was found between them. It appears to be a rare example of a spontaneous mutant thrown up during an outbreak caused by a well known stable subtype—in this case A1/ Liverpool.

The strains isolated during 1957 pandemic showed as usual a close similarity antigenically ; but fell into two groups sharply divided by their sensitivity to nonspecific inhibitors. They differed also in serological behaviour though not so distinctly as in their sensitivity to inhibitors. These groups are being examined to see how far they represent variants or mutants. The biological differences between them were found to be retained without any change up to 6 egg passages.

Studies are in progress on the effect of inoculating mixed virus suspensions allantoically, varying the combinations of strains, dilutions of inocula, intervals of incubation and other details. The results obtained have proved interesting. Some of them have confirmed the findings of previous workers like Hirst and Gotleib regarding phenotypic mixing in the first generation ; but even inocula in high dilution of such mixtures have continued to give strains comdining the properties of the parents, up to 4th passage. The variants are being passed further in eggs to see whether changes occur after the 10th passage as in Hirst's experiments.

It has also been found that when mixtures containing the A2 strains characterized by poor growth in eggs and low HA titres, along with those growing rapidly in eggs like A1/Liverpool/51 and A1/NED/56 are inoculated, the A2 strains seem to overgrow the old ones, despite the excellent egg adaptation of the latter. Some of the variants thus obtained from the strain PAR at 3rd and 4th passage levels are characterized by a remarkedly rapid growth manifested by HA titres averaging about 1,600 and going up to maximum of 4,096 and in a few instances to 8,192 by the usual methods. If the profuse growth characteristic can be retained, the main difficulty experienced with this strain for vaccine manufacture in 1957 will disappear. Its immunizing value is under study.

243. Study on the incidence of influenza in Calcutta and the suburbs, as gauged by isolation of the virus from suspected patients and determination of the presence of influenza antibody in general population under Dr. D. N. Sen Gupta at the R. G. Kar Medical College, Calcutta.

The object of this Enquiry is to reveal the real incidence of influenza, by demonstration of the virus from clinical cases and the determination of influenzal antibody by haemagglutination inhibition test.

During the year under report, attempt has been made to isolate the virus from altogether 27 cases, clinically resembling influenza. In 7 out of these cases, the embryo died within 24-36 hours. In rest 20 cases no virus could be isolated. (Method of isolation same as in previous year).

Samples of sera were collected from patients, in acute and (1-3 days), in convalescent phase (10-13 days) of illness. Paired samples were obtained only in 13 cases, whereas the number of convalescent sera collected were 132. On 19 cases, samples of sera only from acute phase of illness were btained. The sera were heated and preserved in merthiolate. Demonstration of antibody was made by haemagglutination inhibition test.

244. Study of biological behaviour of the strain of variola virus on tissue culture under Dr. R. N. Shukla at the Medical College, Nagpur.

As a first step all "12 strains" collected were passed through the chorio-allontois of hen's eggs from time to time with a view to ascertain their viability. These were preserved suitably under—20°C. deep freeze.

Preliminary experiments undertaken to establish cell lines locally from human amnion and chick embryo proved successful.

Method.

(a) The collected strains were maintained in the laboratory by serial passages over the chorio-allontois of hen's eggs. The material was preserved in 40 per cent glycerol and stored at—20°C. deep freeze.

(b) Human amnion was collected under aseptic precautions in a 250 c. c. ground glass stoppered bottle. The bottle contained P.B.S. 200 units of penicillin and streptomycin.

The material was immediately transferred to the laboratory where after proper cleaning and removal of the clots, it received three washes in an Erlenmeyer flask in Hanks solution. A portion of the material was removed for testing its sterility. The flask was also left over night at room temperatured. after the material was resuspended in medium with 5 per cent human serum and 0.16 per cent sodium carbonate and antibiotics to test if it was free from bacterial contamination.

The material was rewashed three times in Hanks and put for trypoinization 300,003 cells per ml. were seeded in 1 ml. quantity in screw capped test tubes. The tubes were incubated at 37°C. : by third day a good confluent culture resulted. (J. Ferguson and J. Tobin, Brit. Med.Jour. 144-146-1938).

(c) Ceil lines fron chick embryo were also established through cover glass methods (Paster R.C. Methods in tissue culture 2nd ed. 1950).

245. Observations on enteric viruses associated with cases of febrile illness and or meningitis and encephalitis in children under Drs. N. P. Gupta and Sharda Paul at the K. G. Medical College, Lucknow.

1. Investigations into the occurrence of febrile illnesses in children with neurological involvement revealed that sporadic cases could be detected all the year round. A total of 41 cases were observed from July 1957 to June 1958 of whom 9 died.

During the months of July and August 1958 an epidemic broke out in Lucknow city and over 270 cases, clinically diagnosed as encephalitis were admitted to the pediatric ward. Sixty-two of these expired within 24 hours of their admission.

Laboratory investigations revealed that all the cases from the two periods under study could be broadly classified into two distinct groups, those showing definite changes in c.s. fluid along with pleocytosis and those without any pleocytosis. Other positive laboratory findings from the epidemic, were polymorphonuclear leucocytosis, lowered blood and c.s. fluid sugar. Bacteriological findings were uniformly negative. Apart from these two groups, 16 cases with other febrile illnesses and 19 healthy children were also included in the study to serve as control.

2. A total number of 154 specimens were collected during 1957-58 and 1,005 specimens during the 1958 epidemic. One to two days old suckling mice and monolayer cultures of monkey kidney epithelial cells (MKEC) grown as stationary cultures were used for the isolation, propagation and identification of viruses. Each infant mouse was inoculated intracerebrally and subcutaneously. Inoculated mice were observed daily for a period of 3-4 weeks for signs of sickness. Inoculated MKEC tubes were observed for a period varying from 8-15 days daily under the microscope for evidence of cytopathogenic effect (CPE). A total of 235 specimens were inoculated into MKEC cultures and 89 specimens into infant mice. Three hundred and sixtyfive litters of mice have been used so far, each litter containing an average of six infant mica. Thirtytwo monkeys were used to prepare monkey kidney epithelial cell cultures.

3. Fourteen cytopathogenic agents, 13 from stool and 1 from c.s. fluid were isolated from 1957-58 cases, while 28 cytopathogenic agents (26 from stool one from rectal swab and one from c.s. fluid) and 14 mouse pathogenic agents (12 from stool, one from serum and one from c.s. fluid) were isolated so far from the 1958 epidemic. Of the 1957-58 isolates, 10 were from cases of encephalitis and/or aseptic meningitis and four from normal healthy children. Ten duplicate specimens from one autopsy were inoculated in tissue culture and four in mice. Only one has so far yielded a probable mouse pathogenic agent.

(a) Neutralisation tests were carried out with 36 cytopathogenic agents so far, against 9 antisera (polio I, II, III; Cox. B. 1-5 and Cox. A9), U.S. gamma globulin and polio monkey sera. Results revealed 5 polio type I, 3 polio type II, 2 polio type III, 3 Cox. B3 and 4 Cox. B5 strains.

The remaining 19 strains could not be neutralised by the sera used and of these 6 were not neutralised even by U.S. gamma globulin.

Of the two strains isolated from c.s. fluid, the 1957-58 strain (P 100) has been typed as Cox. B5 while the 1958 strain (P. 587) which is not neutralised by U. S. gamma globulin remains untyped. This strain was found to be very slow growing. After 7-8 passages only patchy CPE was seen, that too after 48-72 hours.

(b) Mouse pathogenicity confirmed by histological examination revealed twelve infant-mouse-pathogenic strains, one of which could be propagated in mice only and showed histopathological lesions characteristic of Cox. A infections.

4. A study of the antibody pattern of the paired sera collected from the cases and their contracts is in progress.

MISC. RESEARCHES

277. Field studies on vital statistics and related health phenomena to amplify and assess the accuracy of vital statistics in a rural Punjab population under Dr. J. B. Wyon and Prof. John E. Gordon at the Indian Harvard Ludhiana Population Study Centre, Khanna.

Five different studies are being carried out in 11 villages of Ludhiana District Punjab to amplify and illuminate vital statistics. These villages are also being used for a population study. Some of the data necessary for the population study, such as births, deaths and census are also used in these five studies on vital statistics,. In addition more data are required for each of the vital statistics studies

1. *Death Study. (Dr. Sohan Singh)*

This is designed to give :

(a) Accurate crude death rates also neonatal, infant and other age specific death rates, as well as rates by caste, and village.

(b) Comparison of death rates obtained in this study with those of the village chowkidars' (watchmen's) records of deaths and Census of India population.

(c) Causes of death according to the International Classification.

(d) Effect of season on death rates and causes of death.

(e) Comparison of causes of death according to this study and the village chowkidars.

(f) Types of medical care used in fatal illnesses in these villages.

2. *Birth study. (Dr. Helen Gideon)*

This is designed to give :

(a) Accurate crude birth rates, and fertility rates by age, caste and village.

(b) Comparion of birth rates from study data and from chowkidars' records of births and Census of India population.

(c) Seasonal distribution of births.

(d) Ratio of live births to still-births and abortions.

(e) Investigation of proneness of special groups of women to pregnancy wastage, by maternal age, parity, and possibly other variables.

(f) Factors which affect the interval between deliveries.

(g) Sex ratio at birth, correlated with factors, such as maternal age.

(h) Period of gestation, and relation to factors such as maternal age.

3. *Obstetric Practice. (Dr. Helen Gideon)*

This is designed to make :

A preliminary description of the practice of village midwifery and results on mother and child :

(a) Prenatal, natal and post natal care in these villages.

(b) Obstetric data, such as presentation of foetus and duration of labour.

(c) Obstetric practice, such as care and cutting of cord.

(d) Complications of gestation, delivery and post natal period.

(e) Maternal deaths.

(f) Congenital anomalies.

4. *Study on mortality and morbidity of children under 4 years of age. (Dr. Ishwari Devi, Dr. Narinder Sobthi and Dr. Hardarshan Kaur)*

This is designed to give :

(a) Accurate death rates, especially neonatal and infant, and rates in the second and third years of life, an idea of quarter-yearly death rates, especially in the first year of life, and sex and caste-specific death rates.

(b) Causes of death, specific to age, sex and village.

(c) Morbidity rates, with diagnosis of illnesses specific to age, sex, caste and village.

(d) Fatality rates of diseases common in young children.

(e) Relation between male and female morbidity.

(f) Ages at which children are immunized against small pox.

(g) Pattern of child feeding, especially to study the influence of weaning on morbidity and mortality.

5. *Accident Study (Dr. Prem Vir Gulati).*

This is designed to give :

(a) Accident rates of major and non-disabling accidents for the village as a whole, and also by age, sex, caste and occupation.

(b) The times, places and seasons at which accidents occur most frequently.

(c) The nature of injuries and permanent disabilities caused by accidents in these villages.

(d) The agents which cause the accidents.

IV. NUTRITION RESEARCH LABORATORIES, COONOOR

STUDIES ON PROTEINS

Nutritive value of proteins.—The essential amino acid content of four green leafy vegetables and the cystine content of a few important foodstuffs were determined in view of paucity of data on Indian foodstuffs.

The favourable effect of supplementation of cereals with pulses and green leafy vegetables on the growth-promoting property of the resulting protein mixture was confirmed in investigations on two millets, viz., thenai (*Setaria italica*) and samai (*Panicum miliare*). Dehusking affected adversely the nutritive value of protein in thenai, samai and jowar while there was no change in bajra.

Investigations on the regeneration of haemoglobin and plasma proteins by mixed vegetable proteins were continued. This regeneration proceeded at a slower rate than that observed with skim milk. Albumin was regenerated more quickly than globulin.

On a balanced vegetable protein diet, make up of a mixture of vegetable proteins, the intestinal synthesis of vitamin B_{12}, as also its utilisation for growth and maintenance of tissue concentration was normal. Figures for serum vitamin B_{12} lend support to this conclusion.

Effect of protein deficiency on Pyridine Nucleotide Metabolism—A study of the effects of protein deficiency on the chain of reactions involved in the bio-synthesis of DPN from nicotinamide and glucose phosphate showed that DPN pyrophosphorylase was perhaps the first to be affected, which might account for decreased concentration of DPN in livers. Species difference in pyridine nucleotide (PN) synthesis by erythrocytes was observed. The species that were unable to synthesise PN had a higher PN content in the blood than those which did. Choline deficiency potentiated the effect of protein on liver and blood in depressing the PN synthesising capacity of the liver.

Minimum protein requirements in pregnancy.—Investigations on pregnant women using the nitrogen balance technique showed that 0.9 g./kg. of body weight per day was the minimum requirement between the 22nd and 28th weeks of pregnancy.

STUDIES ON IRON METABOLISM.

Studies on the effect of phytate on iron absorption revealed that with intakes of about 20 mg. of iron per day, the body is in equilibrium with respect to iron even when phytate content is high as in diets based on cereals and pulses. Investigations on iron lost through sweat gave the average values of 0.99 and 0.31 μg. per c. c. in 'cell rich' and 'cell free' sweat respectively.

STUDIES ON VITAMINS.

Vitamin A.—The level of protein intake influences the rate of dep-

letion of liver stores of vitamin A in experimental animals kept on vitamin A—free diets. Rats on high protein diet had a higher requirement for vitamin A than on low protein diet.

Clinical investigations.

Protein malnutrition.—A low protein—low fat diet was found insufficient to induce changes in the metabolism of hepatic ferritin in rats for which the superimposition of a high fat intake was necessary. In monkeys, on the other hand, low protein—low fat diets alone brough about changes in the liver resulting in the formation and release of active ferritin in circulation which was preceded by the expansion of thiocyanate space. On nutritional rehabilitation, ferritin disappeared from blood before thiocyanate space returned to normal.

Dietary fat, serum cholesterol and atherosclerosis.—The changes observed in monkeys on prolonged feeding of diets containing different fats brought about the following changes in serum cholesterol. Hydrogenated fat caused a larger rise but that on coconut oil was even greater. Replacement of a part of the hydrogenated fat by niger seed oil led to a much smaller increase. Corn oil and groundnut oil did not cause a significant rise. Phospholipid concentrations ran parallel to cholesterol concentrations.

Effect of dietary fats on blood coagulation.—Butter, hydrogenated vegetable fat and coconut oil could be placed in descending order with regard to their effect on whole blood clotting time. Only butter produced a significant reduction in stypven time.

Human serum—.The changes in serum cholesterol observed as a result of different dietary fats were not reflected in serum cholesterol or squalene.

Pathology.

(a) Periportal fatty livers were seen on diets containing 6 to 10 per cent proteins of vegetable origin. Such fatty changes were seen in animals even when choline in diet was adequate. Superimposition of choline deficiency tended to produce a more generalised fatty change. Mensenchymial changes have not been seen in periportal fatty livers.

(b) Cytosiderosis in liver varying from mild to severe was observed in protein deficient monkeys with or without oedema.

(c) A study of normal endochondral calcification in rats revealed that calcium and phosphate were deposited simultaneously in contradistinction to the phenomenon seen in low phosphorus rickets where phosphatic mineralisation was found to precede the deposition of calcium during healing.

Dietetics.

An assessment was undertaken of the relative value of three methods

of diet surveys, viz., (i) oral questionnaire, (ii) three day weighment of foodstuffs, and (iii) weighment of food actually consumed by individual members.

No significant differences were found in the intake of calories as ascertained by any of the above three methods.

FIELD INVESTIGATION.

Dietary habits of tribals.—The dietary habits, infant feeding and weaning practices amongst tribal people in the Nilgiris were studied.

Diet and Nutrition Survey in Ankola (Karwar District).—A comprehensive diet and nutrition survey carried out in Ankola Taluk (Karwar District) revealed widespread incidence of anaemia, the incidence being particularly high in women. Vitamin deficiencies in infants and children were almost comparable in incidence to that observed in the South Indian survey reported earlier. The incidence of glazed tongue was higher among the adults, especially women.

Anaemia amongst plantation workers in Nilgiris.—Anaemia was a major problem in children and women among the families employed in a tea estate. Hookworm infestation was not a predominant factor in the case of anaemia in women. Diet surveys among this segment of population showed that the iron intake was 21.5 mg. per day with the range 14 to 34 mg. The anaemia in children responded to treatment with iron as well as with protein, though supplementation with both iron and protein effected rapid improvement.

SECOND FIVE YEAR PLAN PROJECTS.

Studies in human lactation.—The chemical composition of human milk in poor Indian mothers (200 samples) was not significantly different from the values for proximate principles reported for human milk from Europe and U.S.A. where mothers may be assumed to be well nourished. However, the concentration of some vitamins was lower.

Notwithstanding a lower intake of dietary calcium, the calcium content of the milk of the mothers in South India was higher than the values reported from other parts of the World. No correlation existed between the iron content of milk and haemoglobin level in blood. Protein concentration rose and calcium content fell during pregnancy.

Protein malnutrition.—Protein malnutrition surveys in Andhra Pradesh and West Bengal revealed a high incidence among young children. The incidence was at its lowest in infants under one year of age.

Feeding trials with protein rich foods.—Various protein rich foods have been tried. All of them showed marked improvement in the nutrition of children. Vegetable protein mixtures were found to be nearly as good as skim milk.

Studies on growth and physical development of Indian children—Information was collected on 5,702 children in Kerala and Madras of heights and weights together with certain anthropometric measurements. The object of the survey was to prepare reference curves of existing patterns of growth in different regions of India.

Chemical composition of Indian foods—A large number of samples of cereals, pulses, beans, fruits and vegetables were analysed for proximate principles, vitamins and minerals with a view to determining the extent of regional variations.

Nutrition in relation to factory environment—Investigations at Ahmedabad and Coimbatore have revealed that the workers were under-weight as compared with the middle and upper class people from the same area. Mild to moderate degree of anaemia was present in them. The incidence of nutritional deficiency states was appreciable.

The energy cost of some important jobs performed in the textile industry was determined in two textile mills at Coimbatore.

V. VIRUS RESEARCH CENTRE, POONA.

The Virus Research Centre, Poona, continued its operations during the period October, 1957 to September, 1958 emphasizing, as in the past, the two aspects of programme, viz., training of personnel and research.

During the year, five workers visited the laboratory for training. Four of these trainees were mainly concerned with virus studies and one with training in entomology.

Work was started on the construction of a three-storey building to increase the available laboratory space. It is hoped that this building will be occupied sometime during the coming year.

Early in 1957 the existence of a disease, caused by a virus related to the Russian Spring Summer Encephalitis (RSSE) virus, was discovered in Shimoga District of Mysore State. This disease has been called Kyasanur Forest Disease (KFD). As a result of investigations on this disease, begun at that time, it was found that the disease was prevalent in humans and monkeys and that ticks of the genus *Haemaphysalis* were infected in nature. Several deaths occurred amongst humans and many deaths took place among monkeys in this region. Antibodies to KFD were also found in local small mammals and birds. As a result of the initial investigation, a programme was formulated for continuing the studies during the ensuing year. In conference with the Mysore State Public Health Department officials, it was considered that the most feasible means of controlling the disease, from the standpoint of human population, might be by vaccinating humans against KFD. As it had been shown that the virus was quite closely related to RSSE virus, it was thought best to employ RSSE vaccine, which had been studied previously and was reported to be both safe and effective.

The vaccination programme was started in January 1958 and has progressed throughout the year. Blood samples were obtained from a considerable number of the vaccinated individuals in order to try and determine the serological response to the vaccine. By the end of the year these studies had not been completed but the information available indicated that there was not a very significant production of complement fixing and hemagglutination-inhibiting antibodies stimulated by the vaccine.

Studies of the disease in humans have also continued, with emphasis primarily on detecting human cases in order to follow the spread of the disease in the area. A single case was diagnosed in October 1957. From January through September (1958), 243 cases in humans with 7 deaths were reported by the Mysore Public Health Department. It is quite possible that many more cases, milder in character, might have been missed. On the other hand, it should be stressed that only a limited number of these reported cases has been corroborated by laboratory diagnosis. At the present time, the number of laboratory-proved cases for the year is 51. The number of deaths attributed to KFD that have been confirmed in the laboratory is one.

The area involved in the 1957-58 epidemic is greater than that recognized in the human epidemic of 1957 when it was noted that human cases originated from villages in an area covering about 70 square miles. The involvement of at least 20 new villages reporting cases this year extends the affected area to about 100 square miles.

Reports of monkey deaths have continued throughout the year. By the end of September 1958 the area reporting monkey deaths comprised approximately 600 square miles. It should be noted here, however, that none of the monkeys which were found dead outside the epidemic area and from which attempts were made to isolate virus, could be proved to have been infected by the KFD virus. The reasons for this are not clear.

Studies were also continued in trying to elucidate the epidemiology of this disease in man and animals. It has been possible to associate virtually all of the nymphal *Haemaphysalis* tick species with recognised and named adults, but the identification of larvae is still not accomplished with the exception of one species. Studies are under way to try to find characters of the larval forms which will permit specific identification, as it is of great importance to establish precisely the identity of the ticks responsible for the transmission of this disease.

Studies are also continuing on the bionomics of these ticks. It has been difficult to obtain authentic records of ticks attacking man and it would appear that the disease in man is an accident. It is thus very important to try and determine the animals or birds responsible for the spread of the disease in nature. Studies along this line have also actively continued and, in addition to monkeys, increasing evidence has been obtained in favour of infection of several species of birds and mammals. The determination of their precise role in this disease is the subject of continued study both in the laboratory and in the field.

Although the study of Kyasanur Forest Disease was the principal activity of the VRC during the year, studies were continued in the Vellore area on the Japanese B Encephalitis (JBE) and related viruses present there. During the year there was no proved cases of JBE in humans. Field studies have been continuing on the bionomics of the mosquitoes presumably involved in the spread of this disease. As these are long-term studies an analysis of the results so far obtained would be premature.

The Virus Research Centre was also asked to collaborate in the study of other epidemics and reports were rendered for these specific studies as the investigations warranted.

VI. BLOOD GROUP REFERENCE CENTRE UNDER THE DIRECTOR, INDIAN CANCER RESEARCH CENTRE, BOMBAY.

Preparation of reagents.—Thirteen donors were immunized with Rh antigen and nine are being regularly followed. About 1500 c.c. of processed anti-Rh serum is available for local use and supply. 750 c.c. of standard Coombs reagent is also now available. Efforts are being made to prepare other antibodies of the Rh system and 50 c.c. of anti-C has already been prepared.

Work on antenatal Rh testing.—9452 women who came at the antenatal clinic of the hospital were investigated for ABO and Rh blood groups. All available cord bloods were investigated for blood groups and further by Coombs reagent. 9 cases of Rh isoimmunization and 10 cases of ABO isoimmunization were detected in this series.

Study on blood transfusion service.—1352 patients were investigated in detail for transfusion reactions, and efforts were made to isolate factors responsible for them. The hospital authorities have agreed to make necessary changes in their current practice so as to reduce the incidence of these reactions. An attempt is being made to develop a simple filter-drip from indegenous material, so as to improve the present widely used method of exposing blood before transfusion.

Biochemical studies.—Blood group substances are now being prepared in sufficient quantities required for isoimmunization and absorption work of the centre. Efforts are being made to prepare standard quality of bovine albumin useful in Rh testing. Starch Gel Electrophoresis is being standardised for the detection of haptoglobins.

Reference work.—With the assistance of the Blood Group Reference Laboratory in London, it has been possible to build a reference panel of 120 persons who are working in the four co-operating institutes. As a result of work done so far, 2 cases of anti-H, one case of rare blood group Ag, several cases of A_3 and one case of strong anti-P have been detected.

Training programme.—The Centre is now in a position to start its training programme for blood bank officers and technicians. Facilities have also been provided to train two fellows of the Indian Council of Medical Research for advanced type of blood group work.

Report on Studies on Twins and Consanguinity up to August 1958.

Study on Twins :

1. In order to obtain an accurate incidence of twinning at birth and proportion of zygosity, records of three big maternity hospitals in Bombay City were examined for a six-year duration (1952-57). The survey included 104,338 births.

2. Thirteen more twin pairs were added to the series bringing the total to 140 twin pairs examined so far. These records were examined critically and revised list of characters has been prepared for a fresh series of twin pairs to be taken up for the refinement of diagnostic criteria for zygosity.

3. Theoretical methods are being tried out to determine the probability of monozygosity or otherwise and to evolve a linear function to classify doubtful cases.

Study on Consanguinity :

1. An opportunity was presented to the unit to study 7,249 marriages in the rural areas of Andhra State for the rates of consanguineous marriages. Exceptionally high rates were encountered in this area.

2. A study has been undertaken in July 1958 in association with Dr. Gopal Rao at Osmania Medical College, Hyderabad, to relate the rates of consanguineous marriages to the rates of still-birth and congenital malformations.

3. A study of 21 cases of leukoderma in 16 Parsi families revealed 5 families with first cousin marriages. This rate was significantly higher than the rate in the community indicating the presence of recessive genes as one of the aetiological factors.

VII. SCHEME FOR THE MAINTENANCE OF STOCK TISSUE CULTURES UNDER DR. (MRS.) K. J. RANADIVE AT THE INDIAN CANCER RESEARCH CENTRE, BOMBAY.

The stock of commercial cell lines HeLa, KB, Hell, Detroit-6 (malignant) and Intestine and Conjunctiva (Normal) have been maintained for regular supply for experiments in Virology. Maintenance procedure is same as reported previously but the synthetic medium is now prepared in the laboratory, thus reducing the expenditure considerably.

I. *Supply service :*

This service has been well organised, and it is possible to supply any of the available strains at short notice. The following cell strains are available at present :

(i) *Commercial :* Malignant : HeLa (Gey)

KB (Eagle)

Hell (Melnick)

Detroit-6 (Stulberg)

Normal : Intestine (Henle)

Conjunctiva (Chang)

(ii) *New strains :* Malignant : (i) HLS_2 (Human Lipogenic Sarcoma)

Sublines (a) HLS_2 (Hm) and (b) HLS_2R (Hm) — Origin-Heterologous Hamster Tumors.

(ii) MFS (Mouse Fibrosarcoma).

Normal : (i) SPG (Human Embryonic Spinal Ganglion)

(ii) SkM (Human Embryonic Skeletal muscle).

Primary HLS_2 line is now 1½ year old and other lines are about a year old and are considered fairly well established. The cell lines are already in use for virus cultivation, at different centres.

The following is a tabular statement of supply service during 1957-58 :

Laboratory	Strain	Total No. Cultures	BME (Litres)	Remarks
G. S. Medical College, Bombay.	MFS HLS$_2$	25	2.8	Cultures supplied for direct experiments in virology.
Haffkine Institute, Bombay.	HeLa HLS$_2$	10	2.4	Cultures supplied for stock maintenance and virus cultivation.
Pasteur Institute of Southern India, Coonoor	KB MFS	6		-do-
Virus Research Centre, Poona.	HeLa	2		-do-

Dr. Pukhner of Polio Institute, Moscow, USSR has requisitioned the new ICRC HLS$_2$ strain through the Indian Embassy for polio Research. It was supplied in September 1958 and was received in good condition.

Since the medium is now prepared in the laboratory, it is becoming possible to meet modest demands of few laboratories. This service can be expanded considerably.

II *Basic studies and Experimental work on new cell lines :*

Cell biology and cytology of new cell lines developed in the department is carefully studied so as to collect all basic information on the cell material before using it for experiments.

Biological and cytological studies on the three cell lines HLS$_2$. HLS$_2$ (Hm) and HLS$_2$R (Hm) have now been completed and the data are ready for publication. HLS$_2$ is a polyploid strain with a stemline number near tetraploid-4n. HLS$_2$ (Hm) is also 4n and HLS$_2$R (Hm) 3n. The strains are very rich in cytological abnormalities.

The cell strain HLS$_2$ is being tested for virus cultivation. The following viruses produce cytopathogenic effect on the cells : (1) Polio I, II, III ; (2) Coxsackie-B-5, (3) ECHO-strain-D-8, Type 7 strain 84, (4) APC Type 2 ; Type 5.

MFS (Mouse Fibrosarcoma)

The cell line MFS, is also studied carefully for its behaviour in tissue culture and its cytology. The data are being analysed for publication.

Biological studies have been undertaken with a view to study nutritive requirements of the cell type. Amino-acid requirements of the strain have been thoroughly worked out by replicate culture method. These studies provided data for preparation of new medium BNM (Basic Nutritive Medium), which contains optimal quantities of amino acids required for this cell type.

Human Embryonic Tissues :

Experiments are in progress to establish strains derived from skeletal muscle and spinal gangeia. Both the types are fibroblast like in their morphology. The new medium BNM prepared for Fb-L cells of MFS, might prove suitable for maintenance of SPG and SkM strains. These human embryonic cell types SPG and SkM are found particularly useful for "*in vitro*" studies on human leprosy.

With the availability of such varied valuable cell material in continuous cultivation it is possible to expand the programme of experiments considerably in the near future. Many experiments on interesting problems in histogenesis, tissue interactions and experimental carcinogenesis are already in progress.

Publications :

1. Propagation on the glass surface of Human Lipogenic Sarcoma, strain HLS_2.
   Kamal J. Randive, C. V. Bapat and Sudha G. Gangal.
   Tissue Cultural Association Annual Meetings, Philadelphia. Abstracts. Abst. No. 40 : 1958.

2. A cell strain of Human Lipogenic Sarcoma cultivated '*in vitro*'.
   V. R. Khanolkar, Kamal J. Randive, C. V. Bapat and Sudha G. Gangal.
   Indian Journal of Pathology and Bacteriology 1 : 8 :-10 : 1958.

3. '*In vitro*' cultivation of an acid-fast Mycobacterium isolated from human Lepromatous Leprosy.
   C. V. Bapat, Kamal J. Ranadive, V. R. Khanolkar.
   Indian Journal of Pathology and Bacteriology 1 : 156-159 : 1958.

VIII. INDIAN JOURNAL OF MEDICAL RESEARCH.

Since 1913, the *Indian Journal of Medical Research*, published under the aegis of the Indian Council of Medical Research, has been edited by the Director, Central Research Institute, Kasauli. It contains original research papers on bacteriology, virus disease, pathology, pharmacology, physiology, biochemistry, nutrition and public health engineering.

Up to 1957, the Journal appeared quarterly, but beginning with January 1958, it has become a bimonthly publication. The arrears of accepted articles, which had accumulated when the journal appeared quarterly, were cleared in 1958. The Journal attracted papers from foreign research workers as well.

A number of changes in the style of printing were affected and these resulted in quite a handsome saving in the cost of production of the Journal.

To attract more subscribers and to bring the Journal to the notice of medical research workers, one hundred specimen copies were despatched to about 40 countries in the world.

The Journal registered a further rise in the subscribers mailing list. We have 229 foreign and 121 inland subscribers, and it is expected that the number of subscribers will increase in the years to follow.

IX INDIAN JOURNAL OF MALARIOLOGY.

Indian Journal of Malariology Vol. XI. No. 4, 1957.

The September and the December 1957 issues, comprising 72 and 152 pages, respectively, of printed and tabular matter, were published during the year under report.

The March and the June 1957 issues, publication whereof was reported last year, contained 126 and 122 pages, respectively. Thus Vol. XI of the Journal comprised 472 pages of printed and tabular matter as against 364 pages of Vol. X.

Besides contributors in India, the Journal continues to enjoy the confidence of research workers in foreign countries as well. Of the 30 papers published in Vol. XI, 3 were submitted, each one by workers from Burma, Indonesia and U.S.S.R.

Indian Journal of Malariology, Vol. XII, 1958.

The March and the June 1958 issues comprising 76 and 80 pages, respectively, have been published.

The September 1958 issue is in the press and the December 1958 issue is in the process of being sent to press.

Distribution.

Requests from institutions in India and abroad are received regularly for inclusion of their names in the Exchange Mailing List, and where the literature published by the parties, is considered sufficiently useful, such requests are invariably entertained. The distribution of the Journal is shown below.

Subscribers.

Foreign countries	..	..	70	
In India	..	..	98	168
Exchange mailing list	..	..	..	114
Free mailing list	..	..	..	111
Voucher copies to advertisers, specimens, propaganda, etc.	..	..	..	25
Malaria Institute of India	..	..	..	62
Balance held by the publishers for sale				120
		Total		600

X. INDIAN COUNCIL OF MEDICAL RESEARCH LIBRARY. CENTRAL RESEARCH INSTITUTE, KASAULI.

The Indian Council of Medical Research Library at the Central Research Institute, Kasauli has 6,927 bound volumes on its shelves. The Library comprises mainly of Journals received in exchange with the I.C.M.R. publication (Indian Journal of Medical Research). During the year 160 Journals were received on exchange basis and 70 periodicals were received free. Ten books were purchased and 25 books received for review in the Indian Journal of Medical Research added to the books section of the Library.

The Library continued to issue a monthly bibliography in subjects of particular interest to the staff at the Central Research Institute, Kasauli.

XI. (a) MICROFILM AND PHOTOCOPY SERVICE UNIT AT THE CENTRAL RESEARCH INSTITUTE, KASAULI.

During the period under review 102 requests for microfilms and photocopies were received, out of which only 77 could be entertained. The remaining requests for which the publications were not available in our libraries, were forwarded to the Bombay unit and other agencies for compliance.

In all 1460 pages of microfilms and 1281 pages of photocopies were reproduced and supplied in the country and abroad in comparison with 1188 pages of microfilms and 524 pages of photcopies supplied in 1956-57.

The photo-duplication services of the council are being given wide publicity through advertisement in the lay and medical press and extensive circulation of informative folders.

The non-availabiliy of photographic-material restricted the activities to some extent this year. It is hoped that with the import of the necessary material for which a licence has since been obtained, and with extensive publicity, the output of work in the following year will show considerable rise.

XI. (b) MICROFILM AND PHOTOCOPY SERVICE UNIT AT THE TATA MEMORIAL HOSPITAL, BOMBAY.

During the period Ist October, 1957 to 30th September, 1958, 5,864 pages were microfilmed and 2,814 pages were photocopied for various medical and scientific institutions and research workers in India. Some requests could not be complied with because ef non-availability of journals, films and photographic papers. The comparative statement of the documentation work done during the year under report and the two previous years is given below :

	Oct. Sept. 1955-1956	Oct, Sept. 1956-1957	Oct. Sept. 1957-1958
Pages Microfilmed	2,975	4,327	5,864
Pages Photocopied	1,297	1,526	2,814

XII. List of scientific papers based on the work of enquiries financed by the Council, published in 1958.

1 **Agarwal, P.S., Chatterjee, K.P. and Banerjee, S.** Nicotinic acid-Tryptophan metabolism in man, *Ind. Jour. Med. Res.* Vol. **46**. p. 431, 1958.

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